The Epidemiology of Malaria in Belize, 1989 -1999

by

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ABSTRACT

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Retrospective analyses were conducted to describe the epidemiology of malaria in Belize over a 10-year period and to determine if environmental factors influenced the incidence of malaria at macro- and micro-scales. The purpose was to contribute to the current body of knowledge regarding malaria transmission in Belize, which may aid in region-specific malaria control efforts.

Malaria data were obtained from the National Malaria Control Program's National Malaria Database. Malaria rates were calculated using the 1991 population census of Belize (Central Statistics Office). Other data were obtained from the National Meteorological Service (Belize), National Center for Environmental Prediction (NCEP), National Weather Service (U.S.A.), Global Land One-Kilometer Base Elevation (GLOBE), National Geophysical Data Center, Land Information Centre (LIC, Belize), village and vector surveys, Ministry of Health (MOH), SPOT and Landsat multi-spectral

images. Most of the data from these sources were collected for purposes other than the specific aims of the studies in this dissertation.

Over a 10-year period, malaria incidence rates varied temporally and spatially; southern and central areas of Belize had consistently higher rates of malaria than northern areas. Toledo District had the highest *Plasmodium vivax* incidence; whereas, Stann Creek District had the highest *P. falciparum* incidence. Malaria incidence was highest during 1993 through 1996. *Plasmodium falciparum* incidence was highest in the transitional months preceding the wet season in Stann Creek.

Vector surveys conducted in villages in the districts of Cayo, Stann Creek, and Toledo indicated that *Anopheles darlingi* was most common and abundant in Stann Creek District whereas, *An. albimanus* was most common and abundant in the other two districts. The epidemiology of *P. falciparum* incidence among very young children in Stann Creek, along with the common occurrence and seasonal abundance of *An. darlingi,* indicates that malaria transmission occurred locally and *An. darlingi* was the likely vector of *P. falciparum* in that district.

Preliminary results indicated malaria incidence differed geographically by season, type of vegetation, and proximity of villages to rivers or streams. Examination of associations between weather and malaria incidence indicated that precipitation was associated with malaria transmission. Higher total rainfall was associated with a higher malaria risk in villages. An assessment of the relationship between rainfall and malaria incidence in microenvironments, represented by districts, indicated that the relationship seen for the country was especially significant in Cayo and Toledo Districts, where higher rainfall increased malaria risk in villages; whereas, the opposite was seen for

Corozal and Orange Walk. Examination of the relationship between vegetation and malaria incidence indicated that more forest cover was associated with higher risk of malaria in villages. This relationship was specifically seen in Belize, Cayo, and Stann Creek Districts.

Environmental risk factors and malaria incidence were assessed in households in San Martin, Cayo District and Red Bank, Stann Creek District. In San Martin, in 1997, proximity of a household to a stream, number of male occupants in a household, and having a history of malaria in a household were predictive of whether a household had malaria. In San Martin, malaria incidence was highest in males, especially in the 11 to 14 and 35 to 39 age groups. In Red Bank, in 1997, having a history of malaria in a household, construction of the outer walls, and the number of females in a household were predictive for malaria in a household. The 0 to 4 year-old age group had the highest malaria incidence in Red Bank.

Vector surveys conducted in 1997 and 1998 in both study villages showed *An. albimanus* was most common in San Martin; whereas, *An. darlingi* was most common in Red Bank. *Anopheles darlingi* and *An. vestitipennis* were also collected in San Martin, and *An. albimanus,* but not *An. vestitipennis,* was also collected in Red Bank in addition to *An. darlingi*. Malaria cases clustered by household in both villages. In San Martin during 1993 through 1998, only three to eight percent of households produced 50 percent or more of malaria cases. Similarly, in Red Bank during 1993 through 1998, only five to 12 percent of households produced 50 percent or more of malaria cases.

Malaria incidence varied by region, year, season, and populations in Belize during the study periods and these variations are linked to differences in environmental

variables. Malaria control efforts might be more effective if environmental variables were accommodated in malaria control planning.

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Chapter 1

INTRODUCTION

General Introduction

Malaria unleashes a significant toll on populations and strains public health resources. Worldwide, malaria causes approximately 500 million cases of disease and 3 million deaths among 2.5 billion people at risk (Malaria Foundation International, 1997 [http://www.malaria.org]). More than 40 percent of the global population living in approximately 90 countries is at risk. The burden of disease is highest in sub-Saharan Africa where mortality from malaria is highest in children less than 5 years of age and pregnant women. Severity of malaria in children is due to a combination of factors (Greenwood et al. 1991). Some risk factors are the dose of sporozoites, level of acquired immunity, genetic factors, host's nutritional status, socioeconomic factors, and parasite features.

Malaria control efforts, primarily through the use of insecticides, have targeted the *Anopheles* vector since it transmits malaria to humans. Setbacks to this approach have been the development of insecticide resistance in vectors from prolonged use, and the diminishment or abandonment of insecticide use by countries due to its prohibitive costs to resource-strained malaria control programs. Human activities such as mining, clearing of forests to build roads, agricultural practices, and irrigation compound the malaria problem by creating breeding sites for mosquitoes. In Brazil, migration of workers and settlers from malaria-free areas to malarious areas contributed to a significant increase in malaria cases (Cruz 1987).

The epidemiology of malaria differs among countries. Therefore, a universal malaria control strategy is not feasible for solving the malaria problems of individual

countries. An understanding of the risk factors of malaria within countries is necessary to develop effective malaria control strategies.

The study site for the research conducted in this dissertaton is Belize. Belize is a malaria endemic country in Central America. Malaria control efforts, utilizing DDT, started with nation-wide residual house spraying in 1957, which continued until 1989 (Bangs 1999). Pressures by international agencies to reduce the use of DDT changed the scope of malaria control efforts in Belize (Roberts et al. 2002b). House spraying occurred intermittently in 1990 and 1991 and ceased completely in most areas of the country during 1993 to 1995 due to a ban on DDT use in the country. A stratified residual house spray program using DDT and deltamethrin was initiated in 1996 due to a drastic increase (more than 64 percent) in malaria cases during the previous three years of minimal malaria control. The re-initiation of a house spray program decreased malaria cases by more than 40 percent from 1995 to 1996. The use of DDT was abandoned in 2000 (D.R. Roberts, per. comm.) and only the expensive insecticide deltamethrin is in use currently. Additionally, the vector control program, previously vertical in structure, has been decentralized since 2001 and individual districts are responsible for malaria control activities.

As changes within the health infrastructure continue in Belize, financial and human resource constraints may divert malaria control efforts; therefore, it is important to understand the epidemiology of malaria within the country to focus control efforts. In Belize, considerable and valuable research has been conducted in understanding the ecology of malaria vectors. The objective of the research conducted in this dissertation is

to contribute a small piece towards the puzzle comprising the picture of malaria transmission in Belize.

Research Objectives

The purposes of this research were to describe the epidemiology of malaria in Belize over a 10-year span; to retrospectively analyze environmental risk factors of malaria at national and local scales; to increase knowledge of malaria transmission patterns; and to aid in area-specific malaria control efforts. The objectives of this research were:

1) To map average malaria incidence rates from 1989 through 1999;
2) To assess the seasonal distribution of malaria incidence by region;
3) To examine correlations of malaria incidence with vegetation cover;
4) To examine correlations of malaria incidence with rivers and streams;
5) To describe the epidemiology of *Plasmodium falciparum* and *Plasmodium vivax* during 1989 through 1999;
6) To describe the presence and abundance of three main vector species in the Cayo, Stann Creek, and Toledo Districts;
7) To assess the correlation of land cover and meteorological data using remote sensing and geographic information system (GIS) technologies, with malaria incidence from 1993 to 1995 in villages of Belize;
8) To assess the association of household and peri-domestic factors with malaria risk in families in 1997 in San Martin, Cayo District, and Red Bank, Stann Creek District; and

9) To examine the history of malaria in households during 1993 through 1998 in households in San Martin, Cayo District, and Red Bank, Stann Creek District.

Historical Review

The disease caused by species of the genus *Plasmodium* derived its name from the Italians in the 1700's. They assumed the disease was contracted from breathing the 'foul air' of stagnant waters and termed it *mal aria*. Although malaria was named in the 18th century, it has affected man since prehistoric times (Bruce-Chwatt 1965). Ancient clay tablets of Mesopotamia, the ancient Indian medical texts Charaka Samhita and Susruta Samhita, and the ancient Chinese medical text, Nei Ching, mention seasonal and intermittent fevers that may have been due to malaria (Russell 1955). The origin of malaria is uncertain. It is hypothesized that malaria originated in Africa and spread to the Mediterranean, Mesopotamia, the Indian sub-continent and South Asia (Bruce-Chwatt 1965). Others (Coatney et al. 1971) hypothesized that humans acquired malaria from primates in the forests of South-East or South-Central Asia and, over a span of 500,000 years, carried it to other human populations and primates in Africa.

The essay *Airs, Waters, Places* by the Greek physician Hippocrates ('the Father of Medicine') in 5 B.C. dispelled the idea that disease was a curse of the gods and described the epidemiology of malaria in ancient Greece (Hippocrates 1948). He noted an association between the presence of marshes, the time of the year, the geographic location of the patient's area of residence, and the occurrence of malaria. He described the clinical course of malaria, noting that malarial fevers differed from continuous fevers and that patients suffered from enlarged spleens and emaciation.

Etiology

The parasitic etiology of malaria was established in 1880. In the 1800's, several physicians noted the presence of malarial pigments in human blood and tissue without realizing they were looking at the organism that caused malaria. The first to recognize the significance of the pigments was the French Army surgeon, Charles Alphonse Laveran, who was examining the blood of a soldier in Algeria at the time of his discovery in 1880 (Russell 1955). He named it *Oscillaria malariae*, the species that now is called *Plasmodium falciparum*. Golgi, in 1885, demonstrated that two species of plasmodia existed in humans; one was quartan with a three-day life cycle and the other was tertian with a two-day life cycle. He noted that both species (*Plasmodium vivax* and *Plasmodium malariae*) lacked crescent bodies and that the multiplication of the parasites led to paroxysms of fever characteristic of malaria. The Italians Pietro Canalis, Angelo Celli, and Ettore Marchiafava showed the existence of the species of plasmodia named *Plasmodium falciparum* by Welch in 1897.

There were many theories as to how humans acquired a malaria infection. These ranged from drinking swamp waters, as noted by Hippocrates, to the writings in the Susruta Samhita (approximately 3000 years ago), referring to acquisition of fevers from the bite of a mosquito. It was not until 1876 that it was scientifically proven that a mosquito harbored a parasite from human blood. Patrick Manson, a Medical Officer stationed in Amoy, China, discovered that mosquitoes transmitted filariasis and theorized about mosquito-malaria transmission. During 1895 to 1898, Ronald Ross, a British army surgeon stationed in India, tested and proved Dr. Manson's hypothesis on malaria transmission by conducting a series of experiments in mosquitoes and sparrows. Over

the next 50 years, the work of several researchers established the erythrocytic and exo-erythrocytic life cycle of plasmodia.

Prevention and Control

In early history, therapy for malaria ranged from the use of potions and magic by Egyptians, hydrotherapy by the Chinese, arsenic and herbs by Indians and Chinese to Hippocrates' prescription of rest, massage, diet, purgatives, hydrotherapy, venesection and emigration from the malarious area. In recent history, a Jesuit missionary, in Peru in 1600, was cured of malaria by the bark of 'fever tree' given to him by a Peruvian chief (Haggis 1941). This marked the advent of a specific therapy for malaria that was used successfully in saving lives in the Americas, Europe, the Indian sub-continent and Africa. The bark of the *Cinchona* tree was used by the Spanish in the New World and then taken to Europe. In 1820, two French pharmacists, Pierre Joseph Pelletier and Joseph Bienaime Caventou, discovered the cinchona alkaloid, quinine, was responsible for curing malaria. During the First World War, the Germans fearing the loss of quinine supply for their troops, initiated research for a synthetic anti-malarial. The search for synthetic agents in Europe and the USA led to the development of plasmochin (pamaquine) in 1926, followed by atebrin (mepacrine/quinacrine), resochin (chloroquine), primaquine, paludrine, chlorguanide, and pyrimethamine.

Prevention of malaria varied over the ages. The Babylonians used precious stones and amulets for protection, while the Greeks and Romans practiced drainage of stagnant waters. The cinchona bark was used prophylactically by many, most notably the British

explorer David Livingstone in his expeditions in Africa. Sir William MacGregor, an administrator in the British colony Nigeria, employed the prophylactic measures of screening of dwellings, swamp drainage, and the drug quinine. Other measures involved the use of 'illuminating oil,' or kerosene, and the use of Paris green dust to kill mosquito larvae. These larvicides were the primary form of mosquito control until the discovery, in 1939, of the insecticidal properties of dichloro-diphenyl-trichloroethane (DDT) by Paul Muller, a researcher for the Swiss dye manufacturer J.R. Geigy Company. Weissman, a co-worker of Muller, first observed the residual killing property of DDT for houseflies. In 1942, A.W. Lindquist suggested residual use of DDT for mosquitoes. Thus began the DDT era in malaria control. Other synthetic residual insecticides developed were benzene hexachloride (BHC), chlordane, dieldrin, and aldrin. Interruption of transmission through DDT residual spraying, spontaneous clearing of parasites within infected populations within three years, and reports of vector resistance to insecticides, prompted the 14th World Health Assembly (WHA), held in Mexico in 1955, to conceive of the global eradication of malaria (Gabaldon 1969). The principle behind residual spraying was "the interception of the vector" (Gabaldon 1969) and not the elimination of anopheline populations. Malaria transmission was interrupted in approximately one-third of originally malarious areas, with Europe and the Americas having the most success. The 31st WHA, in 1978, discarded the idea of global malaria eradication and adopted a more comprehensive strategy of localized malaria control, which encompassed chemotherapy, personal protection, and community-based vector control.

***Plasmodium* Life Cycle**

The *Plasmodium* life cycle involves two hosts, the vertebrate and invertebrate. The cycle within humans has two phases: exo-erythrocytic and erythrocytic. Four *Plasmodium* species infect humans: *P. vivax, P. falciparum, P. ovale,* and *P. malariae.* Malaria infection begins with a bite from an infected female *Anopheles* mosquito. Sporozoites, the infective stage of plasmodia, are released from the salivary glands of the mosquito into the capillaries of human subcutaneous tissue. Within 30 to 45 minutes of entry into the human bloodstream, the sporozoites migrate to the parenchymal cells of the liver, which marks the beginning of the exo-erythrocytic phase of the plasmodial lifecycle. In the liver, the sporozoites divide and mature into a schizont. The exo-erythrocytic phase may take 6 to 15 days depending on the human malaria parasite species. The mature schizonts rupture and release merozoites. In *P. vivax* and *P. ovale,* some of the schizonts may become dormant in the liver as a hynozoite, which may stay in this stage for months or years after infection and reactivate to produce a relapse of malaria.

The merozoites enter erythrocytes in the blood stream and begin the erythrocytic phase of the malaria life cycle. Within 48 or 72 hours after entry, merozoites are released with the rupture of erythrocytes. The released merozoites infect other erythrocytes. The cycle continues in the blood stream until the host's immune response or chemotherapy controls the cycle. The morbidity and mortality from malaria is due to the erythrocytic cycle of the malaria parasite's life cycle.

Some merozoites may become gametocytes, the sexual stage of the plasmodial lifecycle. If an anopheline bites a human during this period of differentiation, the

gametocytes picked up in the bloodmeal mature in the stomach of the mosquito into macrogametes and microgametes, analogous to female and male parasites, respectively. Fusion of the gametes results in ookinetes, which penetrate the midgut epithelium and form an oocyst in the outside wall of the gut. The nucleus of the oocyst divides and forms sporozoites. Rupture of the oocyst releases the sporozoites into the mosquito's body cavity. Sporozoites then travel in the hemolympth to the salivary glands. Sporozoites in the saliva can infect the human when the female mosquito next blood-feeds for egg production.

The developmental cycle in the mosquito or the extrinsic incubation period of the plasmodia is called the sporogonic cycle and is approximately 7 to 18 days after gametocyte ingestion. Its duration depends on external temperature, humidity, and the anopheline species. The optimal temperature for sporogony is between 20^0C to 30^0C and optimal relative humidity is 60 percent. High relative humidity increases the lifespan of the anopheline and enables an infected mosquito to be infective to humans for a longer period than if the environment were dry.

Anopheles **Life Cycle**

There are four stages in the life cycle of a mosquito: egg, larval, pupal, and adult stages. An adult female *Anopheles* mosquito blood feeds primarily on warm-blooded animals and/or humans to obtain the necessary amino acids required for egg production. Generally, a female takes blood within 24 hours after emerging from the pupal stage. The number of blood meals required by a female for egg production depends on the age and species of the mosquito. Depending on the mosquito species, blood meals may be

taken inside a house (endophagy). After ingestion, the digestion of blood leads to maturation of ovaries during a phase called the gonotrophic cycle. The length of the gonotrophic cycle is influenced by the mosquito species and weather conditions. During the gonotrophic cycle, the female rests within a structure such as inside a house (endophily) or in the outdoor environment (exophily).

The female mosquito lays her eggs (‘oviposits’) on a water surface within two to three days after a blood meal. The oviposition site, or larval habitat, is usually dependent on the anopheline species and is influenced by environmental factors such as pH, salinity, light, shade, and vegetation.

The eggs hatch within 48 hours after deposition. The duration of the larval stage depends on environmental conditions. There are four larval stages, followed by a pupal stage. The pupal phase may last two to four days. The male adult mosquito usually emerges before female. The lifespan of an adult mosquito depends on environmental conditions such as rainfall, humidity and temperature. Rainfall increases relative humidity and both, rainfall and increased relative humidity are associated with increased longevity of adult mosquitoes.

Transmission

Malaria transmission results from complex interactions of four factors: the *Plasmodium* parasite, the *Anopheles* vector, the human host, and the environment. The transmission dynamics of malaria in a geographic area can be expressed by an index, the Entomological Inoculation Rate or EIR. The EIR is a product of the Human Biting Rate, HBR, and the Sporozoite Rate (Bruce-Chwatt 1980). The HBR is the number of female

Anopheles bites per person over a fixed period. The sporozoite rate is the proportion of female *Anopheles* mosquitoes of total female *Anopheles* collected and dissected or analyzed by enzyme-linked immunoassay (ELISA), with sporozoites in their salivary glands. In 1952, MacDonald introduced a transmission model relating the EIR to gametocyte carriers in the human host population (Macdonald 1952). The survival rate of the vector and the length of the sporogonic cycle influence the EIR in this model.

Additionally, he proposed that the basic reproductive rate, R_o, is an index of the stability of malaria transmission. The basic reproductive rate[1] is the number of secondary infections arising from one infected human host given a lack of pre-existing malaria immunity. R_o is proportional to the duration of infection in the host, the HBR, and the average number of human bloodmeals taken by an infected mosquito. For disease transmission to persist in an area, R_o should be more than one (i.e., more than one individual should be infected from one infected host). Similarly, for malaria to be eliminated from an area, R_o, should be less than one.

As the equations for calculating R_o and EIR indicate, malaria transmission and stability are dependent on vector biology, behavior and ecology. Meteorological variables such as humidity, rainfall and temperature influence the duration of the extrinsic incubation period, vector distribution, vector density and vector survival (Onori et al. 1980).

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[1] $R_o = \frac{a^2mcbe^{-\mu T}}{\mu r}$ a, vector biting rate; m= ratio of vectors to host; c, host to vector transmission coefficient or the proportion of bites by vectors on infected hosts that result in infected vectors; b, vector to host transmission coefficient; μ, vector mortality rate; T, the extrinsic incubation period; r, the rate of recovery from infection by the host

Malaria in the Americas

A subject debated among historians was whether malaria existed in the Americas prior to Columbus' attempted expedition to the Far East in 1492. The historians Gualberto Arcos of Ecuador and Jaramillo-Arango of Colombia (Jaramillo-Arango 1950) believed malaria pre-dates Columbus' arrival in the Americas. Facts cited as evidence include the pre-Spanish use of cinchona bark for fevers by the native people of Peru and the Asian origins, where malaria long existed, of the native people of the Americas. Malaria was endemic in western Canada in 1880 but decreased thereafter (Faust 1949). The vectors probably responsible for transmission were *Anopheles quadrimaculatus* and *An.freeborni* (Boyd 1941). Other authors believe that in the United States, as well as Central America, malaria established itself when African slaves were brought to the Carolinas. The vectors responsible for transmission were the same as those in Canada (Faust 1949). Malaria was highly endemic in the South at the time of the civil war. Returning troops to the northern and northeastern United States increased malaria in these areas as well. Heavy use of larvicides, building of drainage ditches, educational efforts aimed at sensitizing the public to malaria control programs, house screening and DDT residual house spraying decreased mosquito populations and malaria transmission in the United States. By 1958, the Global Malaria Eradication campaign eradicated malaria in the United States and Canada (Garcia-Martin 1972).

In the early 20th century, malaria was highly endemic throughout Central America and the Caribbean with the exception of highland and desert areas. Vector species responsible for transmission were thought to be *An. albimanus*, *An. aquasalis, An. bellator, An. darlingi*, *An. aztecus*, *An. pseudopunctipennis*, and *An. hectoris* and *An.*

vestitipennis (Faust 1949, Kumm 1941). At the end of the 20th century, PAHO's Report on the Status of Malaria Programs in the Americas reported active malaria transmission in 21 out of 37 member countries (PAHO 1998). The 21 countries were Argentina, Belize, Bolivia, Brazil, Colombia, Costa Rica, Dominican Republic, Ecuador, El Salvador, French Guiana, Guatemala, Guyana, Haiti, Honduras, Mexico, Nicaragua, Panama, Paraguay, Peru, Suriname, and Venezuela. Brazil reported the highest number of malaria cases followed by the Andean countries. However, French Guiana, Guyana and Suriname reported the highest annual parasitic indices (API) of 78 to 264 per 1000 population (at moderate or high risk). Furthermore, in 1998, *P. falciparum* was the predominant species causing malaria in the three countries as well as the Dominican Republic (PAHO 1998). The mortality rate due to *P. falciparum* cases has dropped from 8.3 per 100,000 population at risk in 1994 to 3.0 per 100,000 population at risk in 1998. Although the prevalence of *falciparum* cases in the Brazilian Amazon region has decreased, in 1997 and 1998, Peru, Ecuador, and Colombia experienced a rise in cases. This shift was attributed to the changes wrought by El Ni_o.

Microepidemiology of Malaria

The epidemiology of malaria differs among countries, regions within a country, human settlements within regions, households within a village, and among individuals within a household (Greenwood 1989). Some of the variations among villages are due to proximity of humans to vector breeding sites (Ghebreyesus et al. 1998, Ghebreyesus et al. 1999, Gunawardena et al. 1998, Lindsay et al. 1995, Subramanian et al. 1991), vector characteristics such as abundance, endophagic behavior, and competency (Grieco 2000,

Roberts et al. 1993, Roberts et al. 2002a, Roberts et al. 2002b, Snow 1987), and housing design that promotes increased human-vector contact (Gamage-Mendis et al. 1991, Koram et al. 1995, Lindsay et al. 1990, Lindsay et al. 1988, Mendis et al. 1990, Schofield et al. 1984). Reasons for variations among households range from the presence of domestic animals near or within houses (Subramanian et al. 1991), behavior of household occupants (Lindsay et al. 1988), and genetic factors of an individual that confer immunity (Flint et al. 1986, Miller et al. 1977).

A vector's host-seeking behavior is influenced by proximity of various hosts. Surveys conducted in Belize have indicated proximity to vector breeding sites influences human landing collections and host-seeking behavior of anophelines (Grieco 2000, Rejmankova et al. 1995, Roberts et al. 2002a, Roberts et al. 1996). Higher numbers of potential malaria vectors were collected at houses within one-kilometer distance of a waterway, especially in the dry season, than distances farther away. The important species approached human habitations for blood meals but varied in their house entry behavior. Higher numbers of *An. darlingi* and *An. vestitipennis* were collected indoors than *An. albimanus, An. apicmacula*, or *An. punctimacula*, which were collected in higher numbers outside the house.

The infection rate of vectors varies by species. ELISA, for circumsporozoite antigen in eight field-collected anopheline species in Belize, indicated four species were positive (Achee et al. 2000). Comparative susceptibility studies of three species of *Anopheles* from Belize with *P. falciparum* (non-native strain) indicated *An. albimanus* was refractory while *An. darlingi* and *An. vestitipennis* were susceptible.

Malaria transmission also varies by households in a community. Preliminary analyses, conducted by Roberts et al., of the occurrence of malaria in four villages during 1989 to 1996 indicated that a mere tenth of households produced more than 50 percent of all malaria cases (Roberts et al. 1999).

Use of Remote Sensing and Geographic Information System (GIS) in Malaria Control

The epidemiology of vector-borne diseases is directly influenced by vector characteristics, which are closely linked to environmental conditions. Spatial and temporal factors such as temperature, rainfall, host population movements, influence vector ecology and parasite distribution. Study of these environmental factors can be limited by the difficulty in reaching inaccessible areas, by the speed in which they change, and by other situations such as natural disasters and civil strife. Remote sensing and GIS facilitate the study of environmental and other epidemiological factors in vector-borne disease.

GIS can be defined as a combination of software and hardware that can be used to collect and manage geographic data. It can integrate topographical maps, satellite images, and aerial photos with attribute data such as demographic and socioeconomic characteristics and disease incidence. It has been used widely to map the distribution of disease and to examine spatial patterns in disease distribution (Beyers et al. 1996, Brooker et al. 2000, Cattani et al. 2001, Cherkasskiy 1999, Kitron 1998, Moncayo et al. 2000, Omumbo et al. 1998). The maps have been used as a tool for developing control and intervention strategies.

GIS has been used in studying many arthropod-borne diseases. The epidemiology of arthropod-borne diseases is directly influenced by vector characteristics. The survival, distribution, and abundance of vectors are closely linked to environmental and climatic conditions such as vegetation, rainfall, and availability of adequate aquatic environments for breeding. GIS, with its ability to integrate and manage multiple geographic and attribute data sources, facilitates the study of the environmental and climatic factors associated with diseases such as malaria, lymphatic filariasis, and onchocerciasis (Bergquist 2001, Sabesan et al. 2000, Seketeli et al. 2002, Sharma et al. 1997, Yamagata et al. 1986).

Passive remote sensing is the measurement, by a sensor, of electromagnetic energy emitted, reflected or scattered from an object. The sensor may be camera film or multispectral scanners on a satellite. A detector on a satellite records the energy and converts it to digital numbers that are represented in a remotely sensed image as picture elements or pixels. Therefore, these pixels represent natural objects on the ground. Pixels having similar spectral signatures can be grouped into classes by the computer (unsupervised classification) and identified by ground verification. Conversely, in supervised classification, a user can classify areas on an image representing known ground cover or 'training sites.' The spectral values of the 'training sites' are then used to group similar spectral values on the image into clusters and therefore define classes, or categories, of ground cover. Commercially available satellite images vary in spatial and temporal resolution, and in channels or the region of the electromagnetic spectrum (EMS) sensed by the satellite's radiometer (sensor) (Hay 2000). The French Satellite pour

l'Observation de la Terre (SPOT) images have a spatial resolution of 10 to 20 meters, a temporal resolution of 26 days, and a spectral resolution of five channels if the image is taken by SPOT-4 ‘s on board High Resolution Visible and Infrared (HRVIR) radiometer. The American Landsat series of satellites produce images of spatial, temporal and spectral resolutions of 15 to 150 meters, 16 to 18 days, and one to eight channels, respectively. Satellite data can be processed to produce surrogates of meteorological data (Hay et al. 1996). For example, vegetation indices, cold cloud duration (CCD) techniques, soil and vegetation moisture, have been used to detect changes in vegetation, rainfall, and land surface temperatures, respectively.

Malaria in Belize

Belize

Belize, known as British Honduras prior to 1973, is located in Central America and bordered by Mexico to the north, Guatemala to the west and south and the Caribbean Sea to the east. Belize covers 22,963 square kilometers (8,866 square miles) in land area. Together, the six administrative districts, Corozal, Orange Walk, Belize, Cayo, Stann Creek and Toledo (Appendix 1) have a population of approximately 230,000.

Belize is geographically, geologically, environmentally, topographically culturally, and racially diverse (Barry 1992, King et al. 1992, Meerman et al. 2001, Wright et al. 1959). Elevation varies from 0 to 20 meters in the marshes and swamp forests of the coastal plain to 1124 meters at the highest peak in the Maya Mountains. Annual rainfall varies from 1200 millimeters in the north to 4000 millimeters in the south. The wet season, also known as the hurricane season, begins in May and lasts through November. The dry season is from January through April. The average annual

temperature is 79°F with minimum temperatures rarely dropping below 55°F. Vegetation types in Belize encompass savanna, mangrove, pine forests, and broadleaf forests. Culturally diverse, Belize is home to many ethnicities such as three Mayan groups, Afro-Belizeans or creoles, Spanish-speaking mestizos, Garifuna, East Indians, and Chinese and Middle Eastern immigrants.

Belize is a malaria-endemic country (PAHO, Roberts 2001). Unofficial reports cited malaria mortality rates of 35 to 91 per 100,000 population during 1929 to 1939 (Faust 1949). In 1939, a considerable proportion (approximately 40 percent) of hospital admissions was due to malaria (Faust 1949). In 1941, two medical officers of the Medical Department of Belize (then known as British Honduras) reported the occurrence of severe malaria in the Stann Creek and Toledo districts (Komp 1940). UNICEF funded and started a DDT house-spraying program in the early 1950's (Brown et al. 1976). As part of the Global Malaria Eradication Campaign, organized nationwide house spraying officially started in 1957 and almost eliminated malaria in Belize. In 1961, only 23 malaria cases were reported. By 1962, Belize was in the Consolidation Phase where elimination of malaria transmission in remaining areas was through case detection, treatment and/or spraying (Brown et al. 1976). However, malaria cases reappeared in 1965 because spraying was stopped, due to a lack of funding. In 1972, 58.3 percent (70,000) of the total population of Belize was living in areas under the attack phase of the malaria eradication program (Garcia-Martin 1972). Spraying declined again in the 1980's and a marked increase in cases was noted from 2,041 cases in 1981 to 4,595 cases in 1983. The Vector Control Program (VCP) continued to use DDT for malaria control until 2000 (D.R. Roberts, per. comm.) when it switched to deltamethrin. Use of DDT

had ceased in most areas (except along the Mexican border) during 1993 to 1995, resulting in increased malaria (Bangs 1999).

The relative prevalence of *P. falciparum* versus *P. vivax* has changed significantly from 1959 to recent years (1998-2000). In 1959, *P. falciparum* cases were three times greater than *P. vivax* cases. The AFI (annual falciparum index per 1000 population) in 1998 was 1.01, while the AVI (annual vivax index per 1000 population) was 10.53. In 1998, approximately 94 percent of the total population was living in areas at risk of malaria transmission (PAHO 1998). Thirty-six percent of the total population was living in areas at high risk of malaria transmission. The MOH reports indicate malaria is generally higher in males and in persons aged 15 to 44 years.

Anopheles Vectors

Three anopheline species are thought to be potential malaria vectors in Belize. Komp reported the presence of *Anopheles darlingi* Root in 1940, and this was confirmed by surveys conducted by Kumm and Ram in the Toledo and Stann Creek districts (Komp 1940, Kumm 1941). Kumm and Ram also reported the presence of *Anopheles vestitipennis* Dyar and Knab, *Anopheles pseudopunctipennis* Theobald, and *Anopheles albimanus* Wiedemann. Grieco, in 2000, conducted a comparative susceptibility study of the three potential anopheline vectors in Belize to a non-Belizean strain of *P. falciparum*. Of the three vector colonies from Belize, *An. darlingi* showed the highest salivary gland infectivity rate (41%) followed by *An. vestitipennis* (9.3%). *Anopheles albimanus* showed no salivary gland infection.

The most widely distributed mosquito in Belize, *An. albimanus,* is associated with cyanobacterial mat and submerged-periphyton habitats (Rejmankova et al. 1993). Females of this species have been collected in greater abundance outside houses than indoors (Roberts et al. 1993). Gabaldon described this vector as a species that lives in coastal areas with low elevation, with its occurrence decreasing as elevation towards inland areas increased (Gabaldon 1949). He noted that mean monthly temperatures of 25^0C or higher may be the preferable habitat for this species along with "sunlit pooled waters" with its distribution having a "direct connection with rainfall, to a much greater extent than *An. darlingi*" (Gabaldon 1949). In 12-hour experimental hut studies conducted in northern Belize, Bangs (1999) reported *An. albimanus* had two peak biting activity periods; two to three hours after sunset and one hour before sunrise (Bangs 1999).

Primarily a riverine anopheline, *An. darlingi* larvae have been found during both wet and dry seasons in shaded or partly shaded patches of floating debris and submersed plants along lowland creek and river margins 11 kilometers or more inland (Kumm 1941, Manguin et al. 1996). Preferred habitats of this species in Central America during the wet season are pooled waters in savannas, and during the dry season, among vegetation on riverbanks where microenvironments of higher humidity are supportive of adult populations (Gabaldon 1949). *Anopheles darlingi* has been observed as having differing distribution by country, and breeding in areas with 1000 millimeters or higher of rainfall. *Anopheles darlingi* throughout its range has been noted as a 'house-haunting species' that preferentially bites humans. This phenomenon has been observed as well in vector studies conducted in Belize (Achee et al. 2000, Roberts et al. 2002a, Roberts et al. 1996).

Preliminary results of 12-hour hut studies, conducted along the Sibun River in Belize, indicate that *An. darlingi* exihibits tri-modal biting activity; approximately 2030 to 2100 hours, slight peak for an hour before midnight, and around 0500 hours. (N. Achee, per. comm.).

Found throughout the year, *An. vestitipennis* is most abundant in the wet season in swamp forest and tall dense macrophyte habitats (Rejmankova et al. 1998, Roberts et al. 1993). At Golden Stream, Toledo, in southern Belize, studies conducted by Grieco (2000) indicated a positive correlation with rainfall. Population densities of both *An. vestitipennis* and *An. albimanus* increased after periods of heavy rainfall (Grieco 2000). Enzyme-linked immunoassay (ELISA) studies testing circumsporozoite antigen for *P.vivax* polymorphs in field-collected anophelines indicated *An. vestitipennis* had higher minimum field infection rates (MFIR) than other vector species (Achee et al. 2000, Grieco 2000). This species, like *An. darlingi*, displays endophagic behavior (Achee et al. 2000, Grieco et al. 2000, Komp 1940, Roberts et al. 1993). A study conducted by Grieco, examining the host-feeding preference of anophelines in southern Belize, indicated that *An. vestitipennis* preferred human blood and attempted to feed throughout the night (Grieco 2000).

Remote Sensing and GIS in Belize

Both unsupervised and supervised classifications have been used in Belize to predict anopheline densities. At high probability sites, using unsupervised classification of SPOT images, analysts predicted the presence of *An. pseudopunctipennis* along the Hummingbird Highway with 50 percent accuracy and *An. albimanus* in northern Belize

with 89 percent accuracy (Rejmankova et al. 1995, Roberts et al. 1996). While using the SPOT image classifications to predict and verify riverine sites for *An. pseudopunctipennis* habitats, Roberts et al in 1993 collected *An. darlingi* larvae for the first time in 50 years in Belize.

Roberts and Rodriguez proposed a sequential approach that specified the steps in conducting studies to use remote sensing to predict the spatial distribution of vectors (Roberts et al. 1994). The approach described five steps: 1) studies identifying environmental variables correlated with vector presence; 2) studies identifying a scale for the environmental factors to be seen on remotely sensed images; 3) analytical studies associating field-collected data with remotely sensed data; 4) use of significant associations from the preceding step to develop a predictive model for vectors; and 5) testing of the predictive ability of the model developed in step four by conducting vector field studies. Several studies conducted have identified environmental variables, detectable on satellite imagery, and have characterized the habitats of the anopheline vectors in Belize (Rejmankova et al. 1998, Rejmankova et al. 1995, Roberts et al. 1993). The sequential approach suggested by Roberts and Rodriguez (1994) can be applied to other components in malaria transmission, for example, the human reservoir.

Satellite data can be used in conjunction with malaria incidence to identify correlated environmental variables. Along with knowledge gained from vector studies in Belize, any correlation found between environmental factors and human malaria would improve the predictive capabilities of a malaria control program and generate new hypotheses for further studies.

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CHAPTER 2

Manuscript 1

Mapping malaria rates and spatial correlations of malaria rates with environmental factors in Belize, Central America

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ABSTRACT

The purposes of this study were to map overall malaria incidence rates from 1989 through 1999 for villages in Belize; to assess the seasonal distribution of malaria incidence by region; and to correlate malaria incidence rates with vegetation cover and rivers in villages, using geographic information system technology.

Malaria information on 156 villages was obtained from an electronic database maintained by the Belize National Malaria Control Program. Average annual malaria incidence rates per 1000 population over 10 years were calculated for villages using the 1991 population census as a denominator. Malaria incidence rates were integrated with vegetation cover from a 1995 vegetation map and with river data from a digital data set.

Mapping malaria incidence over the 10-year period in the study villages indicated the existence of a spatial pattern: the southern and central areas of Belize had consistently higher rates of malaria than northern areas. Examination of the seasonal distribution of malaria incidence by month over 10 years indicated that a statistically significant difference existed among districts and among months ($p<0.05$). Spatial

analysis of malaria incidence rates and vegetation of Belize showed villages with high malaria rates having more broadleaf hill forests, agriculture land, and wetland vegetation types (i.e. SWF-seasonally waterlogged fire-induced shrubland of the plains). Statistical analysis of malaria incidence and spatial analysis of river distributions in Belize determined the high 10 percent malaria incidence villages in central and southern Belize to have more rivers within two kilometers of the center of a village and a statistically significant correlation between proximity to rivers and villages (Spearman's $\gamma = -0.23$; $p<0.05$), especially in Stann Creek District (Spearman's $\gamma = -0.82$; $p<0.05$).

Examination of the distribution of malaria during 10 years indicated transmission varied among geographic areas and among seasons. Additional studies are needed to examine, in more detail, the association between environmental and meteorological factors and malaria transmission. Additionally, the role of *An. darlingi* in malaria transmission in Stann Creek needs further study since, of the three main vectors in Belize, *An. darlingi* has been found associated with rivers.

KEYWORDS: Malaria; GIS; Belize; mapping; environment [factors]; vegetation [environment]; rivers [environment]

INTRODUCTION

A geographic information system (GIS) is a computerized system utilized to process and manage spatial data. GIS is capable of integrating topographical maps, satellite images, and aerial photos with attribute data such as demographic and socioeconomic characteristics and disease incidence. The system has been used widely to produce maps of disease distribution and spatial patterns in disease distribution (Beyers et al. 1996, Brooker et al. 2000, Cattani et al. 2001, Cherkasskiy 1999, Kitron 1998, Moncayo et al. 2000, Omumbo et al. 1998). These maps have been used as tools for developing control and intervention strategies.

In this study, GIS was used to map malaria incidence rates for villages in Belize. The country of Belize, which is divided into six administrative districts (Appendix 1), is geologically, environmentally and topographically diverse (King et al. 1992, Meerman et al. 2001, Wright et al. 1959). Elevation varies from 0 to 20 meters in the marshes and swamp forests of the coastal plain to 1124 meters at the highest peak in the Maya Mountains. Annual rainfall varies from 1200 millimeters in the north to 4000 millimeters in the south. Generally in Belize, the months of June through November are considered the wet season and January through April constitute the dry season. December and May are transitional months where rainfall occurs but not for prolonged periods as in the wet season. Vegetation types in Belize encompass savanna, mangrove, pine forests, and broadleaf forests.

Three anopheline species thought to be potential malaria vectors in Belize have larval habitats characterized by a specific vegetation type. The most widely distributed mosquito in Belize, *An. albimanus* is associated with cyanobacterial mat and submerged-

periphyton habitats (Rejmankova et al. 1993). *Anopheles darlingi*, primarily a riverine breeder, has been found during both wet and dry seasons in shaded or partly shaded patches of floating debris and submerged plants along creek and river margins (Manguin et al. 1996). Found throughout the year, *An. vestitipennis* is most abundant in the wet season in swamp forest and tall dense macrophyte habitats (Rejmankova et al. 1998, Roberts et al. 1993).

The epidemiology of arthropod-borne diseases is directly influenced by vector characteristics. The survival, distribution, and abundance of vectors are closely linked to environmental and climatic conditions such as vegetation, rainfall, and availability of adequate aquatic environments for larval habitats. The aquatic habitats are particularly important for mosquito-borne diseases. GIS, with its ability to integrate and manage multiple geographic and attribute data sources, aids in the study of the environmental and climatic factors associated with diseases such as malaria, lymphatic filariasis, and onchocerciasis (Bergquist 2001, Sabesan et al. 2000, Seketeli et al. 2002, Sharma et al. 1997, Yamagata et al. 1986).

The purposes of this study were to map average annual malaria incidence rates for 1989 through 1999 for villages in Belize, to assess the seasonal distribution of average annual malaria incidence rates by region, and to correlate malaria incidence rates with vegetation cover and rivers in villages. We conducted the study by creating and analyzing a GIS composed of topographical maps of Belize, a 1995 vegetation map, a rivers/streams digital data set, malaria cases from 1989 through 1999 for 156 villages and the 1991 national population census.

METHODS

Using a geographic information system (GIS) created for 213 villages in Belize, this retrospective study assessed the spatial and seasonal distributions of average annual malaria incidence rates per 1000 population from 1989 through 1999 and their correlation with vegetation and rivers and streams in the villages of Belize. Malaria incidence was mapped for every village having census information. The type of vegetation in villages was examined using a 1995 vegetation map for Belize. A digital data set produced by the Land Information Centre (LIC) in Belize provided information on the presence of rivers/streams in villages.

Malaria cases during 1989 through 1999 for the study villages in Belize were obtained from the Belize Ministry of Health's National Malaria Control Program's (NMCP) electronic database. This database was initiated in 1989 for surveillance and malaria control purposes. Malaria case information was incomplete for 1991. Therefore, 1991 malaria data were omitted from analyses. Malaria case information entered in the database was gathered from weekly reports sent by each of the six administrative districts (Corozal, Orange Walk, Belize, Cayo, Stann Creek, and Toledo) in Belize. The weekly report contained demographic information and date of diagnosis of all patients positive for malaria through microscopic examination.

Reports in the districts were generated by malaria surveillance activities conducted in each village within the district. The surveillance consisted of either active, or passive, case detection. In passive surveillance, villagers sought malaria diagnosis, through blood film examination, and treatment from a volunteer health collaborator (VC) in the village. Personnel from the Vector Control Program (VCP), during periods of high

malaria cases, conducted active surveillance in villages by visiting and taking blood films of householders of malaria-positive patients with fever. In both active and passive surveys, the Ministry of Health's district medical laboratory's microscopists examined the blood films. Additionally, all malaria positive films were sent to the central MOH laboratory microscopists for confirmation.

Malaria incidence per 1000 population was calculated for 1989 through 1999 by using the 1991 national census for villages in Belize as denominator data. It was assumed that the entire population in each village was at risk for malaria. The 2000 population census for the study villages was unavailable when this study was conducted. A comparison by the Central Statistics Office of Belize between the 1991 national census and preliminary results of the 2000 census indicated there was an overall 2.7 percent growth in population per year in the country. Average annual, and monthly, malaria incidence rates were calculated for each village and each district. First calculating the malaria rate for 10 years and subsequently calculating the annual incidence yielded the average annual malaria incidence rate for each village. Similarly, calculating the 10-year monthly rate first, and subsequently the annual monthly rate yielded the average monthly malaria incidence rate per 1000 population per year over 10 years.

The geographic location of each village was determined through use of topographical maps. Two sheets of 1:250,000 topographical maps of Belize were electronically scanned in four sections. These maps were then geo-referenced and joined using PCI version 6.2.2 software. The coordinates of each village were obtained by digitizing the location of the village on the maps in PCI software. A vector file of all towns was created and exported to ArcView as a coverage. The attributes of each

village, such as malaria case and census information, were joined to the village coverage using ArcInfo software version 7.2.1.

The average annual 10-year malaria incidence rates for all villages were sorted in ascending order to obtain the high and low 10 and 30 percent malaria incidence villages. The 16 villages with the highest malaria incidence rates, and the 16 villages with the lowest malaria incidence rates, were used to represent the high and low 10 percent of malaria incidence villages, respectively. The 47 villages with the highest malaria incidence rates, and the 47 villages with the lowest malaria incidence rates, were used to represent the high and low 30 percent of malaria incidence villages, respectively. The high and low 30 and 10 percentiles of malaria incidence villages were mapped in ArcView.

Average annual malaria incidence rates per 1000 population for the 10-year period were calculated by district, and graphed by month, to assess seasonal distribution. Differences in average annual malaria rates among districts and differences in average monthly malaria incidence rates were compared using the PROC GENMOD command in SAS version 6.12.

In this study, we used the vegetation map produced in 1994 (published in 1995) by the ecologists Iremonger and Brokaw that showed actual vegetation, cultivated and urban areas (Appendix 2). Iremonger and Brokaw based their map on potential vegetation[1] and soil maps produced by Wright et al. in 1958, satellite imagery, and in depth information on certain local areas (Brokaw 2001). They used a hierarchical system

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[1] Cultivated areas are omitted from display in a potential vegetation map. Instead, potential natural vegetation is illustrated as an indicator of agricultural potential.

that started with three general categories (forest, scrub, and herbaceous), and were further divided to produce 51 vegetation types (36 forest, 9 scrub, and 6 herbaceous).

Each village was given a two-kilometer radius buffer in ARC/INFO. A buffer of one kilometer was chosen to represent the maximum flight range of the *Anopheles* mosquito, and an additional one-kilometer radius was given as an estimate of the size of a village. The village buffers and the vegetation map were integrated using the UNION command in ARC/INFO. Descriptive statistics of the vegetation present within the village buffers were calculated in ARC/INFO. Total area in square meters for the high and low 10 percentile of malaria incidence villages was plotted by vegetation type. Additionally, total area and percentage of vegetation types in villages were calculated by district.

To assess the correlation of malaria incidence with rivers in Belize, we used a digital data set of rivers purchased from the Land Information Centre (LIC), Belize (Appendix 3). The LIC produced the data set by digitizing rivers and streams using 1:50,000 and 1:250,000 Belize topographical maps. To assess the distribution of rivers in high versus low malaria incidence villages, two-kilometer buffers of high and low 10 percent malaria villages were integrated with the rivers data set using the INTERSECT command in ARC/INFO. Distances from the center of all study villages to the closest river were calculated using the NEAR command in ARC/INFO. At both national and district levels, correlations between distances to rivers from village centers, and average annual malaria rates, were calculated using Spearman's correlation (SPSS version 11.0 for Windows).

RESULTS

Only villages with malaria, census, and geographic location information were selected for mapping of malaria distribution for the years 1989 through 1999. The topographical maps had the locations of 213 villages in Belize (Figure 1). Table 1 describes the locations of the digitized villages by district and inclusion criterium. The study included 156 villages and excluded fifty-seven villages due to lack of population census information. The excluded villages had no malaria cases over the 10 years in the study period. A higher percentage (32 to 38) of the excluded villages were located in Belize, Cayo, and Stann Creek Districts.

Figures 2 and 3 display the villages with the high and low 30 and 10 percentile average annual malaria incidence rates, respectively. In both figures, a spatial pattern is seen in in distribution of high and low malaria incidence villages in the study. The villages with higher malaria incidence rates (top 30% and 10%) were located in southern (Toledo and Stann Creek Districts) and western Belize (Cayo District). The villages with lower malaria incidence (low 30 % and 10%) during 10 years were located in the northern areas of Belize (Corozal, Orange Walk and Belize Districts).

Graphs 1- 2 and Table 2 depict the magnitude and seasonal distribution of malaria incidence rates by region (district). Among all districts, Toledo had the highest average annual malaria incidence from 1989 through 1999 (Graph 1, Table 2). During the study period, Cayo, Stann Creek, and Toledo Districts had higher average annual malaria incidence than Corozal, Orange Walk, and Belize Districts (Table 2). Average monthly malaria incidence varied significantly in magnitude among districts and among months ($p<0.05$). The high malaria incidence districts seen graphically in Graphs 1 and 2,

confirm the spatial distribution of malaria incidence rates seen in Figures 2 and 3. Average monthly malaria incidence for 1989 through 1999 was highest in August in Toledo District and in June in Stann Creek District.

Graph 3 depicts the total area of vegetation types within two-kilometer buffers of villages with the highest and lowest 10 percentile of malaria rates during 1989 through 1999. Villages with the highest malaria rates had higher total area of agriculture land, broadleaf forests, and seasonally waterlogged fire-induced shrubland of the plains (SWF) within two-kilometer buffers of villages. Villages with the lowest malaria rates had higher total area of mangrove forests, needle-leaf forests, seasonal swamp forest, tall herb wetland communities, urban development, and water within two-kilometer buffers of villages. The vegetation map illustrated water bodies such as lakes, lagoons, and other contained water bodies but not rivers or streams. Iremonger and Brokaw broadly characterized seasonal swamp forests (SWF) and tall herb wetland communities as wetland communities. Coastal communities included mangrove forests, while forest and scrub communities included needle-leaf and broadleaf forests. Wetland and coastal communities primarily were located in the coastal plains of Belize.

Table 3 describes the total area and percentage of vegetation types in villages by district. Villages in Corozal, Cayo, and Toledo Districts had 70% or more agriculture land than other vegetation types within two-kilometers of the village center. Villages in Belize District had more lowland needle-leaf moist forests (19%), seasonal swamp forests (11%) and urban area (7%) than villages in other districts. Villages in Stann Creek District had more SWF (26%) than villages in other districts. Cayo and Toledo Districts had more broadleaf forest than other districts had (13 and 16%, respectively).

Figure 4 displays a map of rivers and streams within two kilometer buffer zones around the high and low 10 percent malaria incidence villages. More of the high 10% than the low 10% malaria incidence villages had rivers within two kilometers of the centers of villages.

Table 2 depicts the correlations, for all study villages, of distances to the nearest river from the centers of villages and the average annual malaria incidence. Generally, when all study villages were considered in the analysis, proximity to a river was significantly correlated with the average annual malaria incidence in a village (Spearman's γ=-0.23; $p<0.05$). In Stann Creek District, proximity of villages to rivers was strongly correlated with malaria incidence (Spearman's γ=-0.82; $p<0.05$).

DISCUSSION

We used GIS technology to explore the distribution of malaria during 10 years among regions within Belize and to preliminarily assess the correlation of malaria rates with ecological factors such as vegetation and rivers or streams. During the 10-year span, we examined malaria rates by month to investigate seasonal patterns.

Mapping malaria incidence rates for 1989 through 1999 for the entire country showed that malaria distribution varied for the six administrative regions in Belize. The top 10% of average annual malaria incidence villages were located in western (Cayo District) and southern Belize (Stann Creek and Toledo Districts). The four districts had higher annual mean malaria incidence over the 10 years than Corozal, Orange Walk, and Belize Districts. Toledo District experienced significantly higher malaria incidence than other regions. The pattern of high malaria incidence in Toledo was seen especially during 1993 to 1995. Vector control efforts, among other factors, may explain the variation seen in malaria incidence among districts. During 1993 to 1995 minimal or no malaria control efforts were in effect. Nationwide residual spraying, initiated in 1957 and intermittent during 1990 to 1991, were suspended in western and southern Belize during 1993 through 1995 (Roberts et al. 2002b). Vector Control Program records for Corozal and Orange Walk Districts indicate villages in the northern districts were sprayed in 1994. The Mexican authorities occasionally provided DDT, for house spraying, to northern districts bordering Mexico during the three-year period (Bangs 1999). We were unable to assess the relationship between house spray data and malaria incidence for each district during the study period since these data were not available. However, the spatial patterns in districts, together with general vector control information for the country and the

distribution of the three main vector species in Belize, suggest that region-specific factors are associated with malaria transmission.

The spatial distribution of the three potential vectors in Belize help explain the variation in malaria incidence among districts and the correlation of malaria rates with different types of vegetation. Each of the three main vector species in Belize, *An. albimanus, An.darlingi,* and *An. vestitipennis* has unique habitats and differs in its vector competency. The lower malaria incidence villages, primarily in northern Belize, had more total area of coastal (mangrove forest) and wetland vegetation (seasonal swamp forest, and tall herb wetland communities). Entomological surveys in northern Belize have found *An. albimanus* and *An. vestitipennis* (Bangs 1999), (Rejmankova et al. 1995), (Rejmankova et al. 1998). In northern Belize, *An. albimanus* larvae are associated with cyanobacterial mats (CBM), or blue-green algae with precipitated calcium carbonate, that are found in marshes (Rejmankova et al. 1993). In comparative susceptibility studies and field-caught specimens, *An. albimanus* showed the lowest infectivity by *Plasmodium* species (Achee et al. 2000, Grieco 2000) and displayed exophilic behavior (Roberts et al. 2002a). In northern Belize, the wetland and coastal vegetation supports *An. albimanus,* which may be the primary vector of malaria in this region. This species' weak vector association may partly explain lower malaria incidence in northern villages and, therefore, the correlation of low 10 percent malaria incidence villages with coastal and wetland vegetations.

Cayo, Stann Creek and Toledo Districts had higher average annual malaria incidence during the study period. Additionally, the high 10 percent malaria incidence villages had more total area of broadleaf forests, agriculture land, and seasonally

waterlogged fire-induced shrubland of the plains (SWF). Analysis of the vegetation types within all study villages (Table 3) indicated Corozal, Cayo and Toledo Districts had 70 percent or more agriculture land within two kilometers of a village and Cayo and Toledo Districts had the highest area of broadleaf forests near the village. *Anopheles vestitipennis* has been shown to preferentially breed in flooded forests and marshes with *Typha* (Rejmankova et al. 1998). Fertilizer run off from agricultural areas has been known to increase *Typha domingensis* (cattails), a type of marsh vegetation (Selby 2001). *Anopheles vestitipennis* larvae and adults have been found in Toledo district in previous entomological surveys (Grieco et al. 2000, Rejmankova et al. 1998, Roberts et al. 1993). This vector species prefers to feed inside houses and had higher minimum field infection rates than *An. albimanus* or *An. darlingi* (Achee et al. 2000, Bangs 1999, Roberts et al. 1993). It has been determined to be an important vector of malaria in Belize (Grieco 2000). The spatial pattern of higher malaria incidence villages and the correlation with broadleaf forests and agriculture coincide with the spatial distribution and breeding habitats of *An. vestitipennis*.

The higher malaria incidence villages had more rivers present within two-kilometers of villages than lower malaria incidence villages. Proximity of rivers to villages was significantly and weakly correlated with malaria incidence in villages. In Stann Creek District, where the correlation was strong, visual examination of digital data set for rivers in Belize indicates the foothills of Stann Creek District have the densest river and stream systems in the whole country. *Anopheles darlingi* larvae have been associated with river habitats in Belize, and adults and larvae of this species have been collected in all six districts (Kumm 1941, Manguin et al. 1996). Field-caught *An.*

darlingi in Stann Creek District during 1994 to 1997 had a statistically significant minimum field infection rate for human *Plasmodium* circumsporozoite protein (Achee et al. 2000). This *Anopheles* species is a competent vector because it is easily infected by malaria parasites, especially *P. falciparum,* and readily enters dwellings to blood-feed (Grieco 2000, Kumm 1941, Roberts et al. 2002a). The higher malaria incidence in Stann Creek District together with the correlation with rivers and malaria incidence, indicate that *An. darlingi* may play an important role in malaria transmission in this district.

Malaria incidence varied significantly by month within the six regions. Average monthly malaria incidence was highest in Toledo in August. Southern Belize has more broadleaf forests, extensive river systems, and gets more rainfall than northern areas. The ecology of this district (and Cayo) supports *An. darlingi*, *An. vestitipennis*, and *An. albimanus* (Grieco 2000, Manguin et al. 1996, Roberts et al. 1993, Roberts et al. 2002a). At two sites in Toledo, Grieco found *An. vestitipennis* and *An. albimanus* populations to be associated with rainfall and river levels (Grieco 2000). In the study, of the three vector species, *An. vestitipennis* had a higher infection rate in circumsporozoite analysis of landing collections. In the same study, *An. darlingi* populations were lower with increased rainfall, river levels and malaria cases, indicating a smaller role of *An. darlingi* most sites in Toledo District. Rejmankova et al. were able to find *An. vestitipennis* larvae during the wet season, and not the dry season, in Toledo (Rejmankova et al. 1998). In Toledo, during times of high rainfall, it may be that environmental or habitat conditions that are associated with high rainfall are the more likely determinants for increased malaria.

We excluded villages with no population census information. These villages had no malaria cases recorded in the National Malaria Database. Though printed in 1987, the maps we used to digitize villages may have been outdated. The first edition of the 1987 maps was originally produced from 1973-1976 1:50,000 maps (Directorate of Overseas Surveys – Government of the United Kingdom). If the localities indeed still exist, then 37 percent of the excluded villages were from Cayo District. In mapping the high and low 10 and 30 percent villages, we may have underestimated the low or no incidence villages in Cayo.

We extrapolated the 1991 population census to calculate mean incidence rates for the entire 10 years of the study. Population data for individual villages for 2000 were not available when this study was conducted. Therefore, we did not account for variations, over time, in population size in villages. As a result, population size used in our study may have overestimated malaria incidence for the administrative regions. However, we were able to examine at trends in malaria incidence by month and by year during the 10-year period of the study, since Belize maintains a database of incident malaria cases. Using GIS technology, we were able to assess the relationship of vegetation and rivers with malaria incidence for the entire country quickly and cost-effectively.

In our study, malaria incidence had temporal and spatial patterns and a relationship existed between high malaria incidence and proximity to rivers and vegetation such as broadleaf forests and agriculture land. Assuming malaria transmission occurred within the village and was not imported, environmental factors such as forest type, cultivation, rainfall, and proximity of rivers might be useful proxy measures to identify presence of a vector species and its role in malaria transmission. Previous

studies in Belize indicate that the presence and abundance of *Anopheles* species are closely related to ecological niches supported by certain environmental factors (Manguin et al. 1996, Rejmankova et al. 1998, Rejmankova et al. 1995, Roberts et al. 1996). An association was found between *An. vestitipennis* and swamp forests (Rejmankova et al. 1998) and the presence of *An. darlingi* and *An. albimanus* near rivers and creeks (Roberts et al. 2002a). In a vector survey conducted in 1993, collection efforts were guided by predictions based on identification of environmental factors using satellite data and topographical maps (Roberts et al. 1996). At high probability sites, using criteria based on proximity of houses to rivers, altitude of house compounds in relation to rivers, and presence of forest cover, the investigators collected the malaria vectors, *An. pseudopunctipennis* and *An. darlingi,* with 50% and 100% accuracy, respectively. Furthermore, in the investigation, *An. darlingi,* which was last found in Belize in 1946, was successfully collected. Clearly, understanding and identifying the relationship of environmental factors with the ecology of the vectors of malaria in Belize would aid in targeting malaria control measures in a timely and cost-effective manner. Additional studies examining the association of environmental and climatic factors with malaria transmission are warranted.

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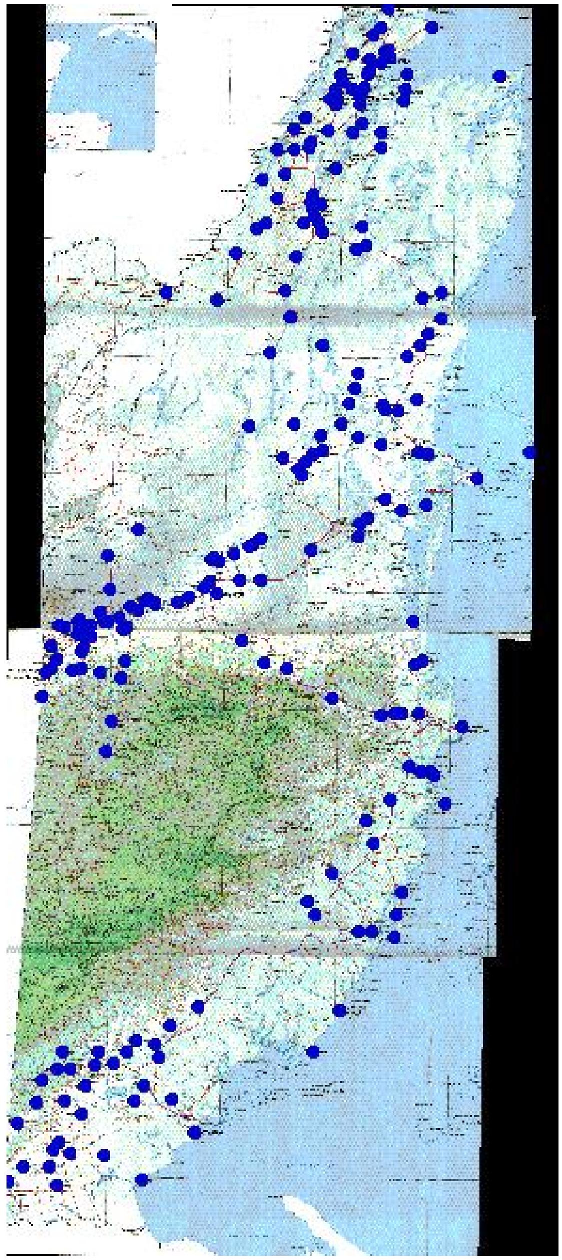


Figure 1: Villages (represented as blue dots) in Belize digitized on 1:250,000 topographical maps, which were electronically scanned in four sections and joined.

Table 1
Study population from 213 villages digitized on 1:250,000 topographical maps

	Villages in study		Villages excluded*		All villages
District	n	(%)	n	(%)	n
Corozal	30	(88)	4	(12)	34
Orange Walk	22	(73)	8	(27)	30
Belize	25	(68)	12	(32)	37
Cayo	34	(62)	21	(38)	55
Stann Creek	16	(67)	8	(33)	24
Toledo	29	(88)	4	(12)	33

*Villages without population census data and no malaria in 10 years were excluded from the study.

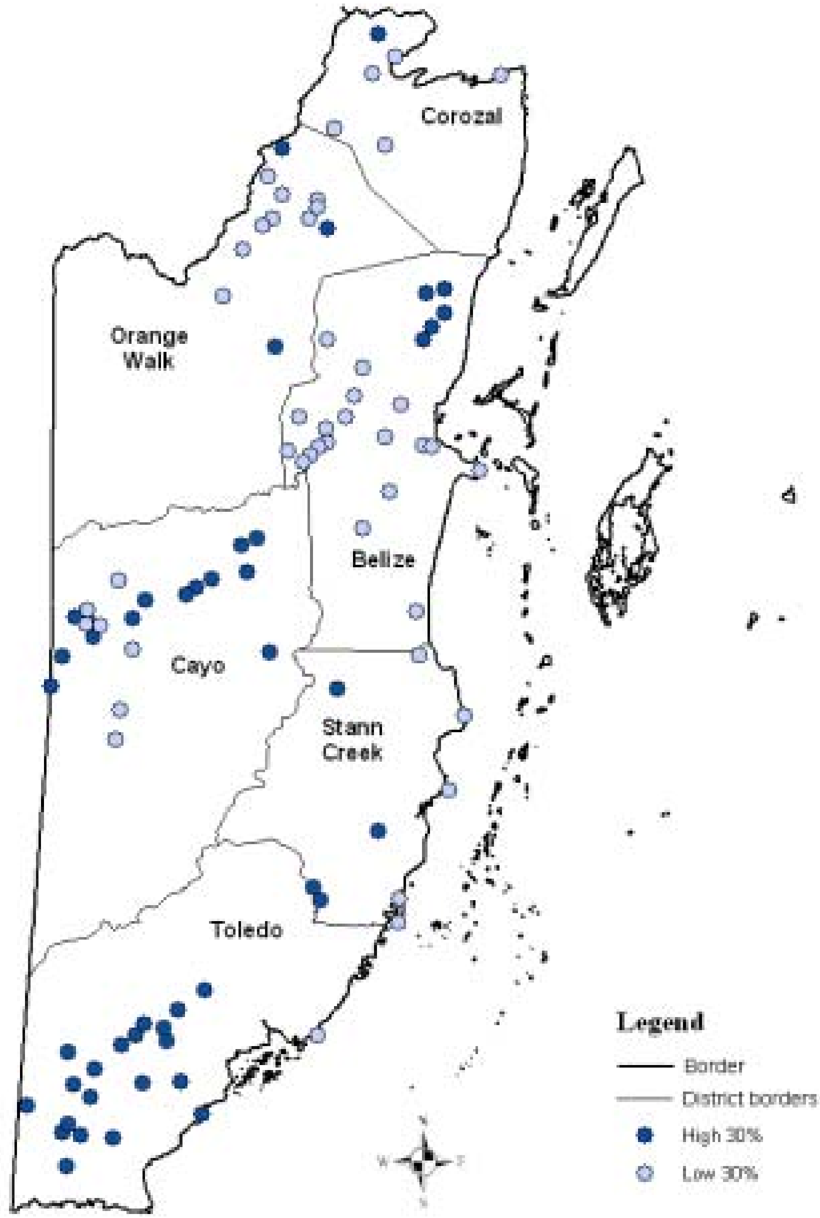


Figure 2: A map of the high and low 30% of average annual malaria incidence villages during 1989-1999 in Belize

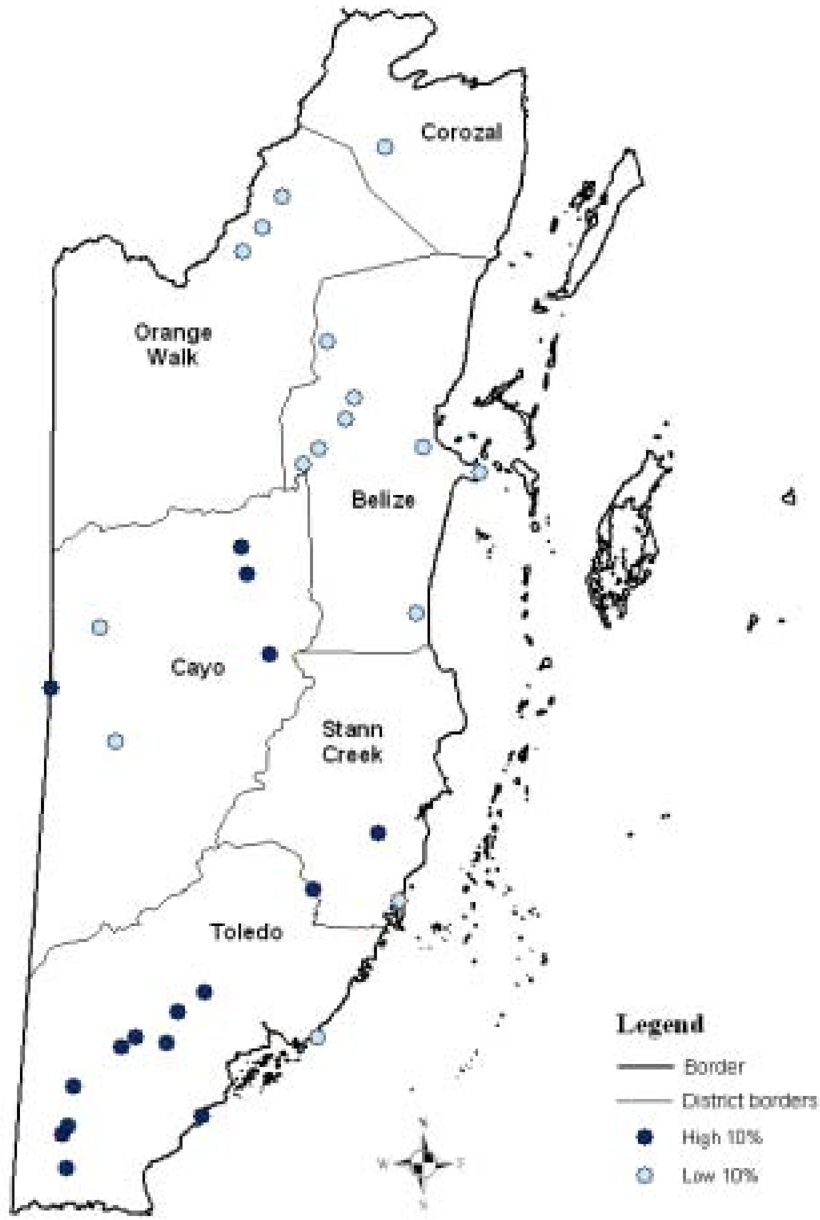
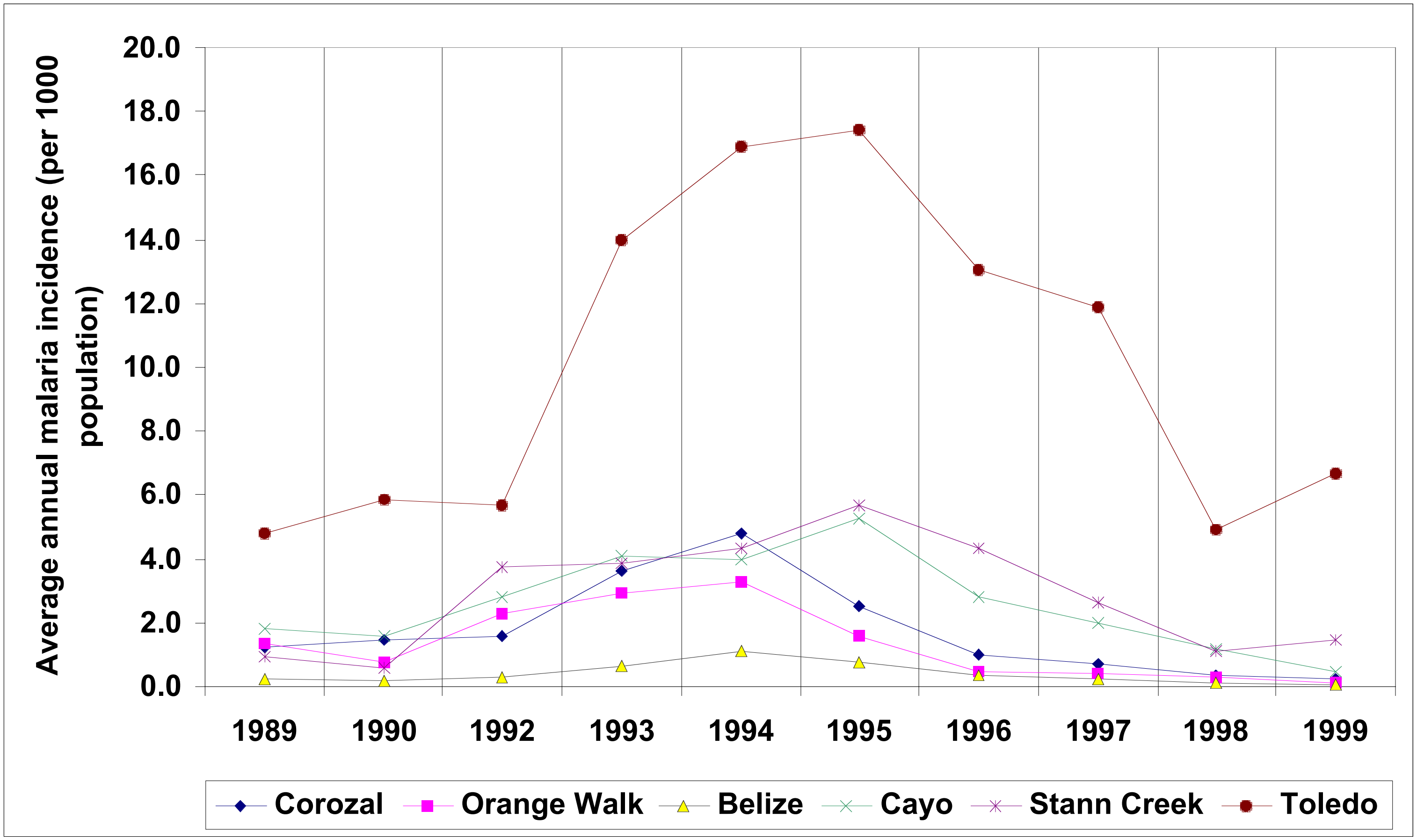
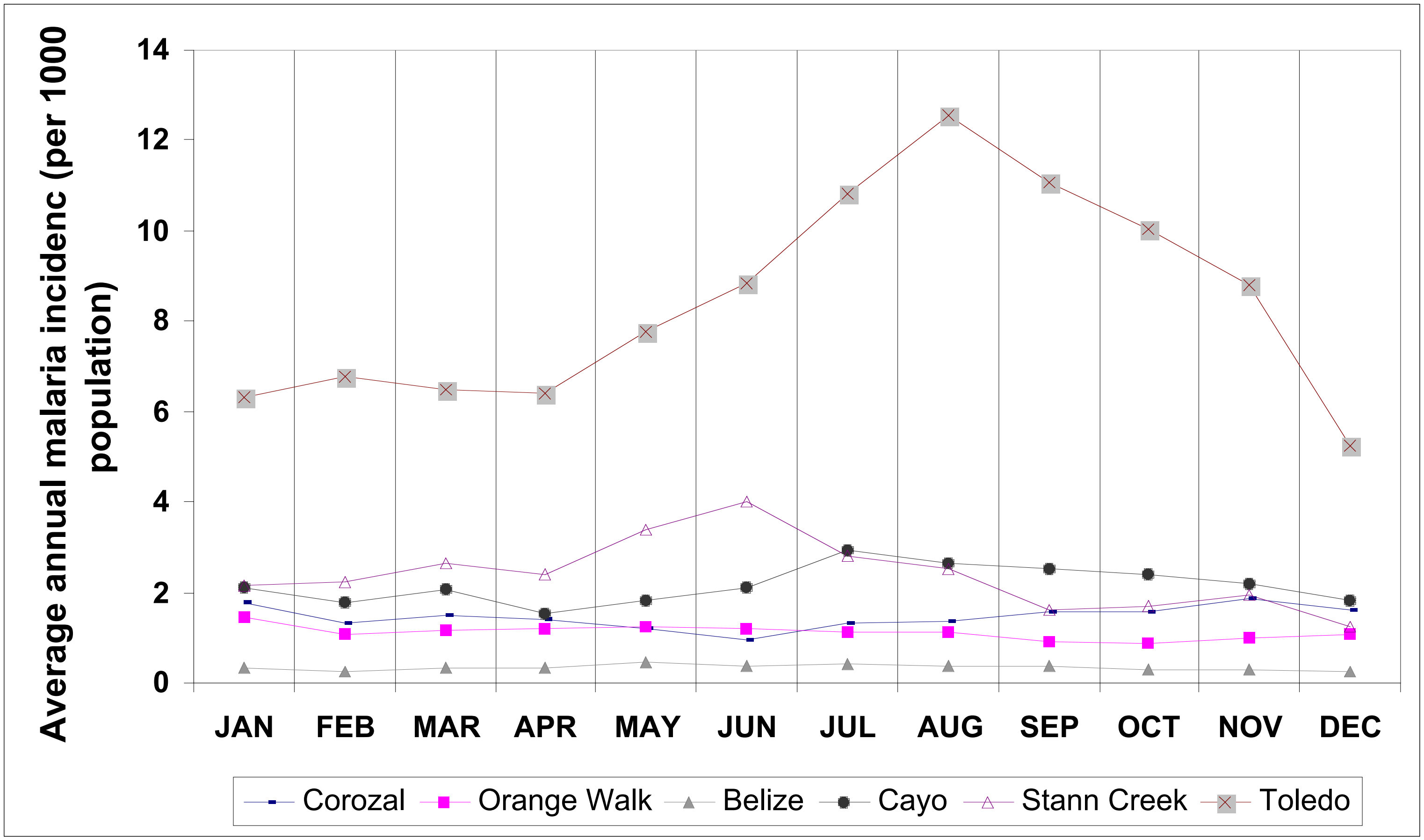


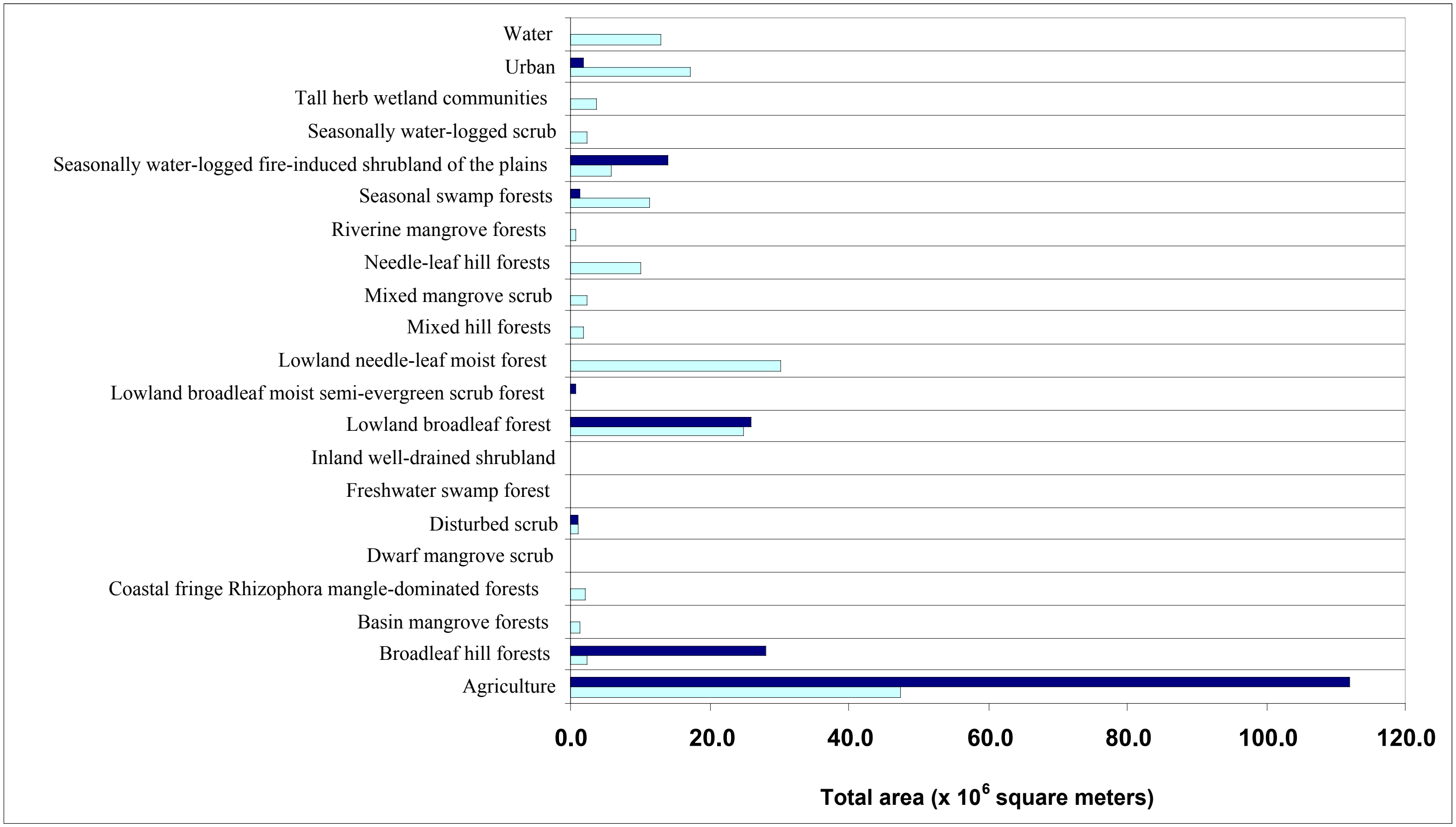
Figure 3: A map of the high and low 10% of average annual malaria incidence villages during 1989-1999 in Belize



Graph 1: Average annual malaria incidence (per 1000 population) from 1989 through 1999 (except 1991) by year for six administrative districts



Graph 2: Average annual malaria incidence per 1000 population by month for the six administrative districts in Belize



Graph 3: Total area of vegetation (square meters) within 2 kilometer buffers around the higher and lower 10% malaria incidence villages. The dark and gray bars represent the higher and lower 10% malaria incidence rates, respectively

Table 2
Descriptive statistics of average annual malaria incidence and proximity of rivers to villages

		Average annual incidence (per 1000 population) during 1989-1999				Distance to rivers from center of the village (meters)					
District	No. of villages	Minimum	Maximum	Mean	S.E.	Minimum	Maximum	Mean	S.E.	γ (Spearman)	p-value
All villages	156	0	97.7	28.5	1.9	1.4	7392.8	1407.1	139.7	-0.23	0.004
Corozal	30	1.4	82.9	23.0	2.7	38.6	7392.8	3532.5	383.7	0.04	0.85
Orange Walk	22	1.7	87.9	21.3	4.5	42.8	3865.8	1320.8	265.0	0.09	0.68
Belize	25	0.3	66.0	14.6	3.9	1.4	3408.4	965.8	208.7	-0.36	0.08
Cayo	34	0.0	77.4	34.0	3.8	6.9	2500.3	469.0	95.5	-0.07	0.71
Stann Creek	16	4.0	93.4	28.1	7.1	16.0	5368.6	1120.4	418.4	-0.82	9.8E-5
Toledo	29	3.6	97.7	45.3	5.0	5.6	5901.2	912.1	258.5	0.08	0.68

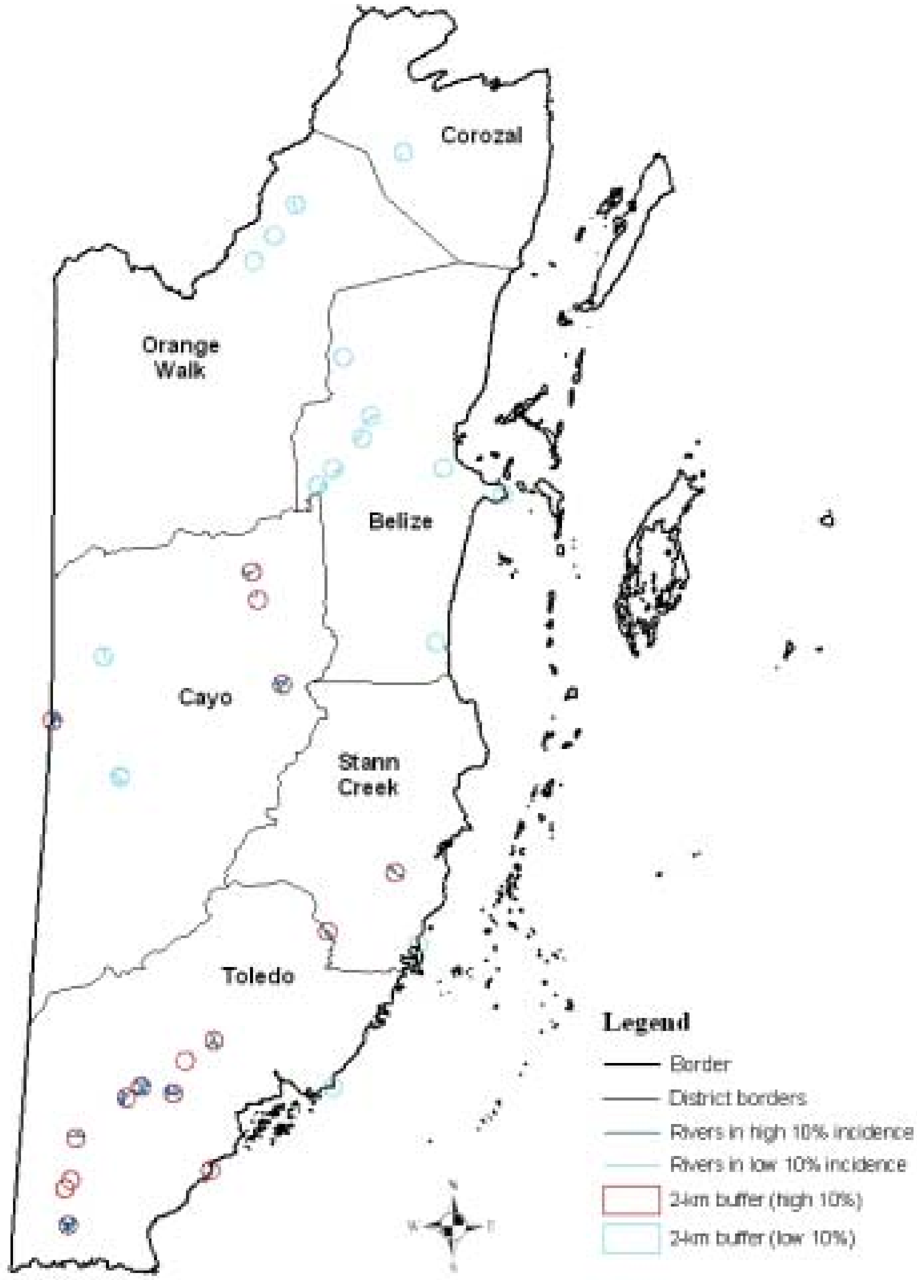


Figure 4: Rivers and/or streams within 2-kilometer buffers of high and low 10% malaria incidence (per 1000 population) villages

Table 3
Total area (square meters) of vegetation within two kilometers of 156 different villages in Belize

Vegetation type	Area (%) Corozal (n=30)	Orange Walk (n=22)	Belize (n=25)	Cayo (n=34)	Stann Creek (n=16)	Toledo (n=29)
Agriculture	190.0 (74)	148.2 (61)	32.6 (11)	246.2 (70)	66.7 (42)	230.9 (71)
Broadleaf hill forests	0	0	4.1 (1)	44.8 (13)	7.1 (4)	51.1 (16)
Basin mangrove forests	0	0	5.7 (2)	0	1.0 (1)	0
Coastal fringe Rhizophora mangle-dominated forests	4.3 (2)	0	0.2 (<0.5)	0	2.1 (1)	2.7 (1)
Dwarf mangrove scrub	0.2 (<0.5)	0	0.9 (<0.5)	0	0	0
Disturbed scrub	0	1.3 (1)	5.5 (2)	6.2 (2)	1.9 (1)	1.6 (<0.5)
Freshwater swamp forest	0	0	0	0	0	0.8 (<0.5)
Inland well-drained shrubland	0	0	0	0.8 (<0.5)	0	0
Lowland broadleaf moist evergreen seasonal forests	8.1 (3)	8.5 (4)	88.0 (30)	27.7 (8)	6.4 (4)	20.5 (6)
Lowland broadleaf moist semi-evergreen scrub forest	1.9 (1)	0	0	0	8.0 (5)	0
Lowland needle-leaf moist forest	0	12.7 (5)	54.4 (19)	2.2 (1)	7.9 (5)	1.4 (<0.5)
Mixed hill forests	0	0	0	1.7 (<0.5)	0	0
Needle-leaf hill forests	0	0	0	15.4 (4)	0	0
Mixed mangrove scrub	0	0	0	0	5.5 (3)	0
Riverine mangrove forests	17.8 (7)	29.2 (12)	5.2 (2)	0	1.1 (1)	0.2 (<0.5)
Seasonal swamp forests	15.4 (6)	19.1 (8)	32.5 (11)	0	1.2 (1)	10.9 (3)
Seasonally water-logged fire-induced shrubland of the plains	0	6.5 (3)	20.7 (7)	1.1	41.5 (26)	3.6 (1)
Seasonally water-logged scrub	0	5.8 (2)	6.9 (2)	6.5 (2)	0	0
Tall herb wetland communities	1.9 (1)	9.2 (4)	3.8 (1)	0	0	0
Urban	9.5 (4)	0	20.9 (7)	0.6 (<0.5)	7.0 (4)	2.5 (1)
Water	8.5 (3)	2.0 (1)	9.5 (3)	0	1.7 (1)	0.1 (<0.5)

CHAPTER 3

Manuscript 2

The epidemiology of *Plasmodium falciparum* and *Plasmodium vivax* during 1989 to 1999 in Belize, Central America

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ABSTRACT

The epidemiologic characteristics of malaria vary within a country. An understanding of the scope and epidemiology of malaria within countries is critical to successful malaria control. In this paper, we describe the epidemiology of *Plasmodium falciparum* and *Plasmodium vivax* infections in Belize, Central America during 1989 to 1999. We hypothesize that infection rates due to these two malaria species differ by region and coincide with the presence and abundance of the three main vector species of *Anopheles* in these regions. Specifically, we hypothesize that the geographic variation in *P. falciparum* infection rates coincides with the presence and abundance of *An. darlingi.*

This retrospective descriptive epidemiological study assessed the distribution of malaria cases and mean annual malaria incidence by species, district, year, age, gender and season in Belize during 1989 to 1999, as well as the distribution and abundance of three primary *Anopheles* vectors. Malaria information was obtained from a national database maintained by the malaria control program in the Ministry of Health (MOH). Vector data were from surveys conducted by a MOH team during the wet and dry seasons of 1996 and 1997 in 32 villages in Cayo, Stann Creek, and Toledo Districts.

Stann Creek District had the highest overall incidence of *P. falciparum* compared to the other districts. *Plasmodium falciparum* incidence was highest in the 0 to 4 year-old age group and the 35 to 44 year-old age group in this district. Furthermore, *An. darlingi*

was collected most commonly in Stann Creek District, which had the highest mean human biting rate of the three southern districts. *Anopheles albimanus* was ubiquitous in Cayo, Stann Creek, and Toledo Districts with index of species abundance (ISA) values of 1.2, 2.8, and 1.5 respectively. A high ISA attests to the mosquito being present and abundant (i.e., common) at most sample sites. *Anopheles darlingi* was the second most common species in the Cayo and Toledo districts (ISA=3.6). In the country of Belize, the total number of cases and the incidence of *P. vivax* were highest in the years 1993 through 1995, while for *P. falciparum* both cases and incidence were highest in the years 1994 through 1996. Toledo District had a significantly higher overall incidence for *P. vivax* than the other five districts. Cayo, Stann Creek and Toledo Districts experienced a greater burden of malaria during 1989 to 1999 than Corozal, Orange Walk, and Toledo Districts. This study supports results from other studies in Belize indicating the importance of *An. vestitipennis* in transmission of malaria in Toledo District.

The epidemiologic characteristics of malaria varied by districts. Local transmission of *P. falciparum* occurred in very young children in Stann Creek District. Additionally, *P. falciparum* infections among working age male adults in Stann Creek District possibly indicates imported malaria, as this region of Belize supports many agro-industries and migrant workers. *Anopheles darlingi* is the primary vector of *P. falciparum* in Stann Creek District. Malaria control programs should accommodate differences among districts in the development and implementation of malaria control strategies. For example, in high *P. falciparum* incidence villages in Stann Creek District, control programs should diligently employ residual house spraying accompanied by malaria education in communities about the benefits of house spraying for malaria control.

KEYWORDS: Epidemiology; malaria; *Plasmodium falciparum*; *Plasmodium vivax*; ISA; *Anopheles darlingi*

INTRODUCTION

In the Americas, 21 of 37 countries are endemic for malaria (PAHO 1998). Belize is an endemic country and has almost 94 percent of its population living in areas at risk of transmission. In Belize, malaria infections are caused by *Plasmodium vivax* and *Plasmodium falciparum.* Transmission is thought to be due to three anopheline species, *Anopheles albimanus, An. darlingi*, and *An. vestitipennis* (Grieco 2000, Roberts et al. 1993). Each species is characterized by specific environmental habitats, which influence their geographical distribution and seasonal abundance.

Anopheles albimanus larvae have been associated with cyanobacterial mats found in marshes of Belize (Rejmankova et al. 1996). *Anopheles darlingi* larvae have been found along river margins among floating detritus and submerged vegetation habitats that are shaded by overhanging trees (Manguin et al. 1996). In contrast to the other two vector habitats, *An. vestitipennis* larvae have been associated with flooded forests and tall dense macrophyte habitats (Rejmankova et al. 1998).

The three species found in Belize differ in vector competency. In a comparative susceptibility study, experimental infections of the three anopheline species from Belize with a non-native strain of *P. falciparum* indicated that *An. albimanus* was largely refractory, while *An. darlingi* and *An. vestitipennis* were susceptible (Grieco 2000). In a circumsporozoite protein (CSP) assay study by Achee et. al. (2000), minimum field infection rates (MFIR) varied among the three species. The enzyme-linked immunosorbent assay (ELISA), for *P. falciparum* and *P. vivax* polymorphs (VK 210 and VK247), was used to analyze eight species of *Anopheles* caught in Belize during 1994 to

1997. The results showed a MFIR of 0.282% in *An. vestitipennis,* 0.271% in *An. darlingi,* and 0.126% in *An. albimanus* (Achee et al. 2000).

Other entomological studies in the Americas also have shown the three species to transmit malaria to humans. Many studies in South America have cited *An. darlingi* as an important vector of malaria (Aramburu Guarda et al. 1999, Camargo et al. 1999, Daniel-Ribeiro et al. 1992, de Arruda et al. 1986, de Oliveira-Ferreira et al. 1990, Rozendaal 1989). Susceptibility studies have shown *An. darlingi* to be readily infected by *P. falciparum* and *P. vivax,* in comparison to other vectors, with high (up to 30 percent) salivary gland sporozoite infection rates (Klein et al. 1991a, Klein et al. 1991b, Klein et al. 1991c, Marrelli et al. 1999, Silva-Vasconcelos Ad et al. 2002). Studies in the Dominican Republic and in Mexico have shown *An. vestitipennis* to be naturally infected with human malaria (Loyola et al. 1991, Mekuria et al. 1991). Indoor collections of *An. vestitipennis* in Belize by Kumm and Ram in 1941 revealed sporozoites in salivary glands (1/41) (Kumm 1941). *Anopheles albimanus* has shown varying infectivity to *P. falciparum* and *P. vivax* based on its phenotype and environmental conditions (Beach et al. 1992, Chan et al. 1994, Collins et al. 1977, Loyola et al. 1993, Ramsey et al. 1994, Warren et al. 1977).

The epidemiology and burden of malaria vary within geographical regions. In South America, French Guiana, Guyana, and Suriname reported the greatest risk of transmission, followed by regions within Brazil (PAHO 1998). Within the four countries, the indigenous peoples of the Amazon Region, and the immunologically-naïve people from other parts of the country who sought work within the Amazon, suffered the greatest malaria burden. *Anopheles darlingi* is a primary vector of *P. falciparum* in the

four countries. Similar to the South American countries, agro-industries in Belize, such as the banana and citrus industries, attract workers from other areas of the country and neighboring Central American countries. It is possible that occupational migration may play a role in increasing malaria transmission in agricultural areas of Belize.

The lifecycle of *P. falciparum*, the species that can cause severe malaria in humans, has a transient exo-erythrocytic stage in the liver, unlike *P. vivax,* which can persist in the liver and relapse if primaquine, a drug that eliminates latent liver stages, is not included in treatment. Therefore, the detection and the treatment of *P. falciparum* cases decrease transmission, unlike *P. vivax* infections where relapses maintain transmission. As local characteristics contribute to malaria transmission within a region, a countrywide malaria control strategy may not reduce the malaria transmission in all areas. An understanding of the scope, and epidemiological profiles, of malaria within localities in a country is therefore critical to successful malaria control.

In this study, we describe the epidemiology of *P. falciparum* and *P. vivax* within Belize during 1989 to 1999. We hypothesize that infection rates due to the two malaria species differ by region and coincide with the presence and abundance of the three main vector species in these regions. Specifically, we hypothesize that the geographic variation in *P. falciparum* infection rates in Belize coincides with the presence and abundance of *An. darlingi.*

METHODS

This retrospective epidemiological study assessed the distribution of malaria cases and average malaria incidence by species, district, year, age, gender and season in Belize during 1989 to 1999, as well as the distribution and abundance of three primary vectors in 1996 and 1997. Malaria information was extracted from a national database maintained by the malaria control program in the Ministry of Health (MOH). Population data were ascertained from the 1991 census conducted by the Central Statistics Office (CSO). Seasonal patterns in the occurrence of malaria cases were compared to daily total precipitation data for 1989 through 1999 acquired from the Belize National Meteorological Service (NMS). Vector data were obtained from surveys conducted by a MOH team during the wet and dry seasons of 1996 and 1997 in villages in Cayo, Stann Creek, and Toledo Districts.

Study area

Belize covers 22,963 square kilometers of land mass and is diverse in its ecology due to varying rainfall patterns, elevations, and soils. The Caribbean Sea borders Belize on the east coast and Guatemala and Mexico lie to the west and north, respectively. The environment is sub-tropical, and limestone and sandy soils support mangrove and marsh swamps in the low-lying areas of the north (Corozal, Orange Walk, and Belize Districts) and along the coastline. Sub-montane and montane vegetation types are located inland (Cayo, Stann Creek, and Toledo Districts) at altitudes ranging from 500 to 1124 meters. Annual rainfall varies from 1,200 millimeters (48 inches) in the north to 4,000 millimeters (160 inches) in the south. Generally, the wet season begins in May or June and lasts through November. The dry season is from January through April. Weather

varies inter-annually. The country, having six administrative districts (Appendix 1), varies by district in population size and in social and economic characteristics. Sugar cane industries primarily characterize the economies of Orange Walk and Corozal Districts; whereas, the banana and citrus industries are located in Stann Creek District. The districts largest in population are Belize and Cayo. The population of the country is approximately 244, 000 (CSO 2000). In 2000, 40 percent of Belize's population was 14 years or younger (CSO 2000).

Malaria database

Malaria cases during 1989 through 1999 for all villages in Belize were extracted from the MOH's electronic database. This database, initiated in 1989 for surveillance and malaria control purposes, is maintained in present day. Malaria case information was not available from the database for 1991. The database serves as a repository of information collected from weekly reports sent to the central unit (Belize District) by malaria control units in each of the six districts of Belize. The weekly report contains demographic information and date of diagnosis of all patients positive for malaria by microscopic exam.

The weekly reports generated by the district malaria control unit are summaries of malaria surveillance activities conducted in each village within the district. The surveillance consists of both activ,e and passive, case detection. In passive surveillance, villagers seek malaria diagnosis, through blood film examination, and treatment from a volunteer health collaborator (VC) in the village. Personnel from the Vector Control Program (VCP) occasionally conduct active surveillance in households where malaria

cases have been diagnosed. In both active and passive surveys, microscopists at the MOH's district medical laboratory examine the blood films. In addition to case information noted on the weekly reports, all malaria positive films are sent to the central MOH laboratory microscopists for confirmation.

Malaria cases

The number of malaria cases per locality was extracted from the national database by year of study, by age, and by gender. A count of localities having one or more cases of malaria of all NMCP localities was determined to calculate the proportion of positive localities by *Plasmodium* species per district (Appendix 4). Initially, cases for both species of *Plasmodium* were categorized into five-year age intervals after infancy (Appendix 5 a,b). Examining malaria cases by age groups allowed us to identify if malaria transmission was higher in certain age groups. More *P. falciparum* (57%) cases occurred in the 20 year-old, or less, age groups. To adjust malaria cases for population size in villages, malaria cases were grouped according to the 1991 population census age intervals. The census age groupings were: 0 to 4, 5 to 14, 15 to 24, 25 to 34, 35 to 44, 45 to 54, 55 to 64, and 65 and over.

Malaria incidence

Plasmodium falciparum and *P. vivax* incidence were calculated for 1989 through 1999 (except 1991, since malaria case information was unavailable). Not all areas listed in the NMCP database as a locality were listed in the 1991 national census. The census only cited the population for villages with 50 or more inhabitants. The proportion of villages with population data, and with one or more cases of malaria, was calculated for each district by *Plasmodium* species. Only the villages surveyed in the 1991 census and

that were malaria-positive were considered in the calculation of malaria incidence. Annual malaria incidence was calculated for each age group and gender in each village by dividing the total number of cases of *P. vivax* and *P. falciparum* by the village population. The annual incidence per village was averaged for 10 years to produce a mean 10-year incidence rate. In calculating incidence, it was assumed that the entire population in each village was at risk for malaria and that population growth over the 10-year period was constant across villages.

The incidence in each district was adjusted to the size of the district for each *Plasmodium* species. Size-adjusted incidence was calculated by the formula:

$$\text{Adjusted incidence rate} = \frac{\text{(Total number of positive villages)}}{\text{(All villages in the district)}} \times \frac{\text{(Number of cases in all positive villages)}}{\text{(population in the malaria-positive villages)}} \times 1000$$

The incidence in positive villages is the malaria cases per 1000 population in a district multiplied by the proportion of all villages in the district that have malaria (by *Plasmodium* species). For each malaria species, calculation of an adjusted incidence rate for each district accomodated for the size of the district (i.e. the number of villages in a district and the population of the district).

The burden of malaria by species was assessed for geographic regions of Belize. The burden due to malaria in a region was determined by calculating the attack rate percent (AR%) per year. We used the AR% to express excess risk experienced by the southern districts (Cayo, Stann Creek, Toledo) when compared to the northern districts (Corozal, Orange Walk, Belize). Generally, the AR%, an incidence measure, is used in investigations of epidemics and is the difference in attack rates of those exposed versus

those unexposed to a specific risk factor (Hennekens 1987). It is useful in identifying the etiology of an outbreak. The AR% for each species was calculated for each study year by subtracting the average incidence per 1000 population in the north from the average incidence per 1000 population in the south.

To calculate malaria incidence for the northern region, the annual incidence for Corozal, Orange Walk, and Belize districts were averaged for each malaria parasite species. Similarly, for the southern region of Belize, the annual incidence for Cayo, Stann Creek, and Toledo Districts were averaged to calculate average incidence for each *Plasmodium* species.

Seasonal pattern

Daily total precipitation data were acquired for all 22 weather stations in Belize, (Appendix 6a). The stations began recording data at different times (Appendix 6b) and were non-functional during a few months of each year over the span of the study period. The weather stations having data for the study period were chosen to represent the precipitation pattern. The weather stations selected were: Corozal – Libertad; Orange Walk – Tower Hill; Belize – Phillip S.W. Goldson International Airport in Ladyville; Cayo – Central Farm; Stann Creek – Melinda Forest Station; Toledo – Blue Creek (Appendix 6a). The NMS precipitation data were averaged across months for 1989 though 1999. Likewise, the cases by malaria parasite species were summed by month across years, and subsequently averaged by district across villages. Seasonal patterns in the occurrence of *P. falciparum* and *P. vivax* were compared to weather data. The malaria cases by *Plasmodium* species were plotted against average daily total precipitation (in inches) by month for the 10-year study period.

Malaria vector data

The vector information used in this study was obtained from surveys conducted by the MOH teams during August 21, 1996 through November 14, 1997. In this study, the months January through June were considered the dry season and July through December the wet season. During the wet season of 1996 and the dry and wet seasons of 1997, the teams conducted indoor and outdoor human landing collections from 1600 to 1800 hours at houses in 32 villages in Cayo, Stann Creek, and Toledo Districts. In each district, we determined the proportion of positive landing collections per district by vector species, the numerical abundance of a species in a district, and the density per two-hour landing collection of the three main vectors.

Frequency in attempted human biting

The proportion of positive landing collections per district by vector species was determined to compare the frequency in human biting by three main vector species. First, the total number of landing collections was determined for Cayo, Stann Creek, and Toledo Districts. Second, for each of the three main vector species, the proportion of positive landing collections was calculated. For each district, the proportion was calculated by dividing the total number of landing collections with one or more adult female captured, either *An. albimanus,* or *An. darlingi,* or *An. vestitipennis,* by the total number of landing collections conducted.

Index of Species Abundance (ISA)

The presence or absence of a vector and its numerical abundance in a district was determined using an Index of Species Abundance (ISA) developed by Roberts and Hsi (Roberts et al. 1979). In calculating the ISA, the vector collections were organized in

rows and columns in a spreadsheet. The species were recorded in rows, R, and the collections (i.e., villages) were recorded in columns, *K*. The data in each column, *K*, were sorted in descending frequency for each *Anopheles* species. After sorting, the data were ranked in increasing order so the highest numbers of a certain species at a village was ranked 1. The formula to calculate the ISA is:

$$ISA = \frac{a + R_j}{K}$$

R_j = sum of ranks in each row

a = (sum of zero cells in K columns) x (c)

c = (largest rank in K columns + 1)

A low ISA value (e.g., 1.0) indicated the mosquito species was present in all collections and most abundant in the district. A high ISA value reflected low numbers of the vector species or its absence in many collections in the district. The number of landing collections per village differed by village and season. To avoid bias due to more sampling in one season than the other, adjustments accommodating the number of landing collections were made prior to calculating the ISA. The adjustment was performed by dividing the number of *Anopheles* collected in each village by the number of landing collections conducted in the village for each season.

Attempted Human Biting Rate

The density of each of the three main vector species attempting to bite exposed adults indoors and outdoors was determined for each season and each district by calculating the human biting rate (HBR). Each district's adult HBR, per person per two hours of landing collection for the wet and dry seasons, was calculated by the formula:

$$HBR_{district} = \frac{\text{Total number of adult female mosquitoes collected during each landing collection}}{\text{Total number of collectors at each landing collection}}$$

Malaria cases in vector-survey villages

The occurrence of malaria cases in the villages surveyed for vectors in 1996 and 1997 was examined for seasonal patterns. Daily total precipitation in inches was averaged by month for 1996 and 1997. Malaria cases by *Plasmodium* species in the vector-survey-villages were summed by month and district. The malaria cases were plotted against precipitation by month.

Statistical analysis

In this study, the GENMOD procedure in SAS version 8 for Windows was used for hypothesis testing. The analysis of variance (ANOVA) in the GENMOD procedure tested the null hypothesis of the equality of the means of the effects of districts, years, months, or age groups on the dependent variable, malaria incidence or cases. A p-value of 0.05 or less indicated that the null hypothesis was rejected and the means among the groups were not equal. Repeated measure and autoregressive statements were used in the analyses. The repeated measure statement was used to indicate that an observation (e.g., a village) was included more than once (ten times for each village during the study period) in the analyses. The autoregressive statement was used to account for a variable's temporal correlation in a village (i.e., the value of a variable for a village that is time-dependent may be closer in value to the subsequent year's value).

RESULTS

Figure 1 shows the distribution of the total number of malaria cases by year during 1989 to 1999. The total number of malaria cases steadily rose from 1989, peaked in 1994, and declined from 1995 to 1999.

Table 1 depicts a breakdown, by species, by year and by gender, of the total number of malaria cases displayed in Figure 1. During the 10 years of the study, *P. vivax* consistently accounted for 90 percent or more of total malaria cases. *Plasmodium falciparum,* during 1989 through 1993, constituted one to three percent of total malaria cases. However, during 1994 through 1996 and in 1998, the proportion of *P. falciparum* cases ranged from four to nine percent. *Plasmodium falciparum* and *P. vivax* cases differed by year of study.

Table 2 shows the distribution of malaria cases by district, by species, and by year. Both *P. falciparum* and *P. vivax* malaria cases differed among districts. Total numbers of cases were highest in the country of Belize during 1993 through 1995 for *P. vivax,* and highest during 1994 through 1996 for *P. falciparum.* During 1997 to 1999, Toledo District accounted for 41 to 54 percent of Belize's *P. vivax* cases. Also in this three-year period, Cayo District contributed 36 to 82 percent of the total *P. falciparum* cases in Belize. In 1992 and 1996, Stann Creek District had the largest proportion of *P. falciparum* cases in Belize (58 and 63 percent respectively).

Table 3 depicts the distribution of localities having one or more cases of malaria within the six districts, by species, and by year. Table 3 includes only those localities (villages) that were listed in the 1991 population census of Belize. For both *Plasmodium* species, the six districts differed in the mean number of malaria-positive localities.

Among *P. falciparum* positive localities, Cayo had a higher mean number of localities than the other five districts. Among *P. vivax* positive localities, the mean number of localities in Cayo and Toledo were higher than the other four districts.

Table 4 presents the incidence per 1000 population for *P. falciparum* and *P. vivax* by district and by year. Mean *P. falciparum* incidence and mean *P. vivax* incidence differed among the six districts ($p<0.05$). During the 10 years of the study, Stann Creek had the highest mean incidence per 1000 population of *P. falciparum* (3.3) followed by Cayo (1.6) and Toledo (1.4) Districts. For *P. vivax,* Toledo had the highest mean incidence per 1000 population (101.5) followed by Stann Creek (32.4), and Cayo (27.7) Districts. Of the three northern districts, Corozal had the highest mean incidence per 1000 population of *P.vivax* (17.5). The incidence of *P. falciparum* was higher in 1996 than in any other year in this study. Figure 2 illustrates the difference in mean *P. falciparum* incidence among the timeframe in this study. Likewise, for *P. vivax,* the mean incidence was higher in 1994 than other years (Figure 3).

Table 5 shows the incidence rate per 1000 population for *P. falciparum* and *P. vivax* by age group, gender and district. For both malaria species, incidence did not differ between males and females. *Plasmodium falciparum* and *P. vivax* incidence, in males and females, were significantly different among districts. Cayo, Stann Creek and Toledo Districts had higher malaria incidence in males and females than did Corozal, Orange Walk and Belize Districts. During the 10 years, the average *P. falciparum* incidence among females was highest in the 15 to 24 year-old age group (1.6), while among males, the 35 to 44 year-old age group had the highest average incidence (2.0). *Plasmodium vivax* incidence was highest among males and females aged 0 to 4 and 5 to 14 years in

Toledo District than among any other age group in that district, and males and females of all ages in the other districts.

Figures 4 (a through d) illustrate the data in Table 5 by gender and *Plasmodium* species. Comparison of *P. falciparum* incidence between females and males among all age groups in all districts indicated the incidence was highest in Stann Creek District. Among females in Stann Creek District, the incidence was highest in the four years or younger age group and among those aged 15 to 24 years (5.0 and 4.6 respectively, Figure 4a). Among males in Stann Creek District, *P. falciparum* incidence was highest in the four or younger age group and those aged 35 to 44 years (5.0 and 5.1 respectively; Figure 4b). Except for the 45 to 54 year age group among males, *P. vivax* incidence was highest in Toledo District followed by Cayo District (Figures 4c and 4d).

Table 6 shows the size-adjusted incidence for *P. falciparum* and *P. vivax* by district and by year. The size-adjusted incidence adjusted for population size and number of villages in each district. Adjusted incidence of *P. falciparum* and *P. vivax* varied among districts. Stann Creek District had the highest adjusted *P. falciparum* incidence compared to the other five districts and Toledo District had a higher adjusted *P.vivax* incidence than any other district.

Table 7 displays the incidence for the North and South of the country for both *P. vivax* and *P. falciparum* by year of study. Incidence differed between the northern and southern regions for *P. vivax* and *P. falciparum* ($p<0.0001$). The South had considerably greater burden of malaria for both species in each of the 10 years. The excess risk or attack rate percent varied from 57 to 97 percent for *P. falciparum* and 61 to 90 percent for *P. vivax*.

Figures 5 (a-f) and 6 (a-f) depict the average malaria cases and precipitation by month for each species and district. Figure 5 displays the seasonal pattern of *P. falciparum* by district. The peak in *P. falciparum* transmission varied by season in each district. Timing of peak case occurrence by district and season, respectively, were: Corozal – January; Orange Walk– dry season and after heavy rains; Belize – dry season and after some rainfall in the wet season; Cayo – wet season with peak transmission in November; Stann Creek – dry season and before heavy rains in the wet season; Toledo – during the height of the wet season. The height in transmission of *P. vivax* differed as well by district and season, respectively: Corozal – after heavy rains and in January; Orange Walk – in January; Belize – before heavy rains; Cayo – during the wet season; Stann Creek – before the wet season; Toledo – after heavy rains.

The villages where landing collections were conducted in 1996 and 1997 are listed by district and season in Table 8. Landing collections were conducted in 12 villages in Cayo District and 10 villages each in Stann Creek and Toledo Districts. Vectors were sampled in both the wet and dry seasons in all three districts. More collections were carried out during the wet season in Cayo and Stann Creek Districts (67 vs. 47 and 65 vs. 42 respectively) than the dry season. In Toledo District, more landing collections were conducted in the dry season (42 vs. 18).

The numbers and percentage of landing collections positive for the three vectors in Belize are shown in Table 9. *Anopheles albimanus, darlingi,* and *vestitipennis* were collected in all three of the surveyed districts, Cayo, Stann Creek, and Toledo. The highest percentage of landing collections positive for *An. albimanus* was in Cayo District

(65 of 114; 57%). *Anopheles darlingi* was collected more commonly in Stann Creek District (47 of 107; 44%), as was *An. vestitipennis* (21 of 107; 20%).

Tables 10 through 13 show Index of Species Abundance (ISA) calculations. Table 10 displays the *Anopheles* collected at each village and the total for each district. *Anopheles albimanus* was collected in the highest numbers (364) in Cayo District. Furthermore, landing collections at Roaring River in the Cayo district yielded 264 *An. darlingi* mosquitoes. *Anopheles darlingi* was most abundant in Stann Creek and Toledo Districts (440 and 47 respectively). San Roman village had the highest numbers of *An. darlingi* in Stann Creek District (119); while in Toledo District, Golden Stream and Indian Creek had the highest numbers of *An. darlingi* of all villages sampled in the district. Table 11 displays the *Anopheles* collected per village adjusted for the number of collections conducted per village per season. Table 12 displays ranks assigned to each *Anopheles* species at each village by district and the corresponding ISA, adjusted only for season, for each district by species. In the three districts, Cayo, Stann Creek, and Toledo, *An. albimanus* had the lowest ISA (1.2, 2.8, 1.5, respectively) followed by *An. darlingi* (3.6, 2.9, 3.6, respectively) and then *An. vestitipennis* (4.8, 4.8, 3.9, respectively). Table 13 represents ISA adjusted for landing collections by season and indoor versus outdoor locations. *Anopheles albimanus* was most common and abundant in indoor collections in Toledo (ISA= 1.8) and Cayo (ISA=2.3), followed by *An. darlingi* (Toledo: 2.9; Cayo: ISA=3.9). The exception was Stann Creek District where in indoor collections *An. darlingi* had the lowest ISA (2.3) followed by *An. vestitipennis* (ISA=2.5). In outdoor collections, as in indoor collections, *An. albimanus* had the lowest ISA in Cayo and

Toledo Districts (1.1 and 2.0, respectively), followed by *An. darlingi* (3.6 and 3.9, respectively).

The mean human biting rates (HBR) for landing collections in the wet and dry seasons of 1996 and 1997 are depicted in Table 14 by district and vector species. The three vector species were caught in landing collections during both wet and dry seasons, except for *An. darlingi,* which was not found in the wet season in Toledo. Comparing the three species among the districts by season, *An. darlingi* and *An. albimanus* were more prevalent in the dry season in Stann Creek (1.9 vs. 1.2 and 0.8 vs. 0.5, respectively). *Anopheles darlingi* and *An. vestitipennis* were more prevalent in the dry season than the wet season in Toledo (0.1 vs. 0 and 0.1 vs. 0.03 respectively). All three vector species had a higher HBR in Cayo District during the wet season than the dry.

Figures 7 (a-f) and 8 (a-f) illustrate the average number of malaria cases for the villages where vector surveys were conducted and average daily total precipitation by month and district for 1996 and 1997. Peaks in average daily total precipitation generally occurred in July for both Cayo and Toledo Districts in 1996 and 1997. In Stann Creek District, most rainfall occurred in November for both the years. As seen previously in Figures 5 and 6 over the 10-year period, the occurrence of malaria cases differed by month and district for 1996 and 1997 in the 32 villages. In 1996, *P. falciparum* cases in 22 villages in Cayo and Stann Creek Districts occurred mostly in July and August, while in 10 villages in Toledo, most cases occurred in July. In 1997, however, the *P. falciparum* case-pattern differed from 1996 for these 32 villages. The most reported cases were seen in October in Cayo, September in Stann Creek, and March in Toledo. In 1996, *P. vivax* cases occurred mostly in August among the 32 villages in the three

southern districts (and July for Cayo). In 1997, the timeframe shifted: most cases occurred at the end of September in the 12 Cayo villages; March in the 10 Stann Creek villages; and October in the 10 Toledo villages.

DISCUSSION

To develop and implement a successful malaria control program, a preliminary investigation of the epidemiology of malaria and an entomologic assessment of potential vectors in geographic regions is necessary. We described the epidemiology of the two species, *P. falciparum* and *P. vivax,* responsible for the malaria burden in Belize during 1989 to 1999. Additionally, we qualitatively and quantitatively assessed vector surveys conducted in the wet and dry seasons of 1996 and 1997 for *An. albimanus, An. darlingi,* and *An. vestitipennis.* Malaria cases in the villages where the vector surveys were conducted and average precipitation by month were examined for 1996 and 1997.

Cayo District had the highest number of *P. falciparum* cases, as well as the highest percentage of villages that had one or more case of *P. falciparum.* However, after adjusting for the number of villages as well the population of villages in a district, Stann Creek District had the highest overall incidence of *P. falciparum* compared to the other districts during 1989 to 1999.

In Stann Creek District, *An. darlingi* was not only most commonly found in indoor and outdoor landing collections in 10 villages (ISA of 2.3 and 2.7 respectively), but also had the highest mean human biting rate of the three southern districts. Of the three main vector species in Belize, *An. darlingi,* had a higher infection rate of *P. falciparum* than *An. albimanus* or *An. vestitipennis* (Grieco 2000). In a comparative susceptibility laboratory study of a non-Belizean strain of *P. falciparum*, *An. darlingi* showed the highest salivary gland infectivity rate (41%) followed by *An. vestitipennis* (9.3%). *Anopheles albimanus*, on the contrary, showed no salivary gland infections. Although the *P. falciparum* strain used in the experimental infections of the three vectors

was not native to Belize, the vector populations in the study were from Belize. Other studies in Belize and in Central and South America indicate that *An. darlingi* is highly endophilic and anthropophagic (Achee et al. 2000, Gabaldon 1949, Komp 1940, Roberts et al. 1987, Roberts et al. 2002, Roberts et al. 1996, Rozendaal 1989).

In Stann Creek District, examination of the average incidence of *P. falciparum* by age and gender spanning the 10 years in the study showed that male and female children four years or younger had the highest incidence (Table 5 and Figures 4 a and b). The younger than four-year-old children may have had different vector exposure than older children and adults. In previous field studies in Stann Creek, the author observed younger children to be less clothed than older children and adults. Consequently, the children may have been exposed to more bites by mosquitos. Furthermore, younger children may have had earlier bedtimes than other family members, and therefore have been easier sources of blood meals for female mosquitos (R.G.Andre, per. comm.). Peak biting periods for *An. darlingi,* in 12-hour experimental hut collections along the Sibun River, indicated attempted human biting was highest around 8:30 to 9:00 p.m., an hour before midnight, and around 5:00 a.m. (N. Achee, per. comm.). The younger children's lifestyle may have placed them in greater contact with vectors and increased their malaria risk. However, the important observation derived from incidence data is that the high incidence among pre-school aged children suggests intra-domiciliary transmission within villages in Stann Creek District. Clearly, indoor transmission is consistent with the endophagic behavior of *An. darlingi.*

During the study period, Stann Creek District was the largest producer, and exporter, of banana and citrus in the country. The two industries hire workers locally, as

well as from other areas of Belize and Central America. The Central American workers are mostly from Honduras, Guatemala, El Salvador, and Nicaragua (PAHO 1994), where the Annual Falciparum Index (AFI) of the four countries in 1998 ranged from 0 to 1.83 cases per 1000 population (PAHO 1998). It is therefore possible that in Stann Creek *P. falciparum* initially was imported from other Central American countries. In addition to the high incidence in very young children, *P. falciparum* was highest also among 35 to 44 year-old males during the 10-year period. The high numbers may reflect cases among the banana and citrus workers in the district. As stated before *An. darlingi* was common in Stann Creek and it is endophagic. Thus, it probably became infected by gametocyte carriers among migrant workers. Once infected, *An. darlingi* is a highly competent vector of *P. falciparum.* The findings of this study combined with the entomologic findings of other studies conducted in Belize, indicate that *An. darlingi* is the primary vector of *P. falciparum* in the Stann Creek District.

Examination of the seasonal patterns of *P. falciparum* and *P. vivax* cases, during 1989 through 1999 in Stann Creek District, showed most cases occurred in May and June just before the onset of heavy rains during the wet season. Belize District exhibited a similar pattern, where cases peaked in numbers prior to the onset of prolonged rainfall. The pattern in both districts may be due to habitat availability for *An. darlingi.* Primarily a riverine anopheline, *An. darlingi* larvae have been found during both wet and dry seasons, in shaded or partly shaded patches of floating debris and submerged plants along lowland creek and river margins 11 kilometers or more inland (Kumm 1941, Manguin et al. 1996, Roberts et al. 1996). Additionally, during May 1993 in northern Stann Creek and Belize Districts, adult *An. darlingi* were collected in human biting collections at all

houses surveyed within one kilometer of rivers or streams (Roberts et al. 1996). As a parallel, in Suriname, heavy rains in coastal areas created habitats in low-lying depressions adjacent to riverbanks, unlike the forested areas of the Amazon, where heavy rains washed out larval habitats (Rozendaal 1992). Likewise in certain river systems in Belize, heavy rains of the wet season may eliminate habitats along river margins, thus reducing adult populations, which in turn may decrease malaria transmission. Many villages in Stann Creek are located in the foothills of the Mayan Mountains. The streams located in these villages can develop fast water currents with the onset of heavy rains. However, the transitional months between dry and wet seasons may create suitable habitats following a prolonged absence of rainfall when river levels are low. Grieco (2000), at Golden Stream, which is located near a small river system in Toledo, observed that adult *An. darlingi* population densities fell appreciably during the rainy season when the river current was fast and river levels high (Grieco 2000). At the end of the dry season at this site, adult populations were highest when malaria cases were lowest. These results indicated that at Golden Stream, *An.darlingi* breeding was associated with rivers and that a vector other than *An. darlingi*, which did not play a significant role in malaria transmission during the wet season when malaria cases were highest, transmits malaria to humans.

In Toledo District, *P. vivax* incidence was highest in males and females aged 14 or younger. The high incidence among the young, as opposed to adults, may indicate immunity acquired with age. For instance, in some countries such as rural areas in the Gambia, symptomatic malaria decreasing with age is characteristic of intense exposure and transmission of a malaria species throughout the year (Clarke et al. 2002).

The seasonal pattern of both species of malaria differed by district. In Toledo District, transmission of both malaria parasite species occurred primarily in the wet season after heavy rains. The same pattern was observed during 1996 and 1997 when vector surveys were conducted. The pattern differed slightly for *P.vivax* in 1997 when most cases occurred in October, later than in the other years. Loyola et al. (1991), at a study site in the Lacandon Forest in Mexico, reported the highest man-biting and *P.vivax* infection rate of *An. vestitipennis* in August when rainfall was heaviest (Loyola et al. 1991). During an 18-month study conducted in Toledo District during 1997 and 1998, Grieco observed that *An. vestitipennis* abundance was positively associated with *P. vivax* and showed the highest MFIR when compared to *An. albimanus* and *An. darlingi* (Grieco 2000). In the study, *An. vestitipennis* was anthropophagic, endophagic and found to bite throughout the night. Other studies in Belize have found sporozoites in the salivary glands of *An. vestitipennis* (Achee et al. 2000, Kumm 1941) and reported its endophilic and anthropophagic characteristics in the Toledo district (Roberts et al. 1993). Additionally, Grieco's study determined that the highest larval abundance and high human landing collections occurred in the wet season when flooded forests provided optimum habitats for this vector. A sampling of habitats in northern Belize and Toledo District in October and November of 1996 and February and March of 1997, found *An. vestitipennis* larvae associated with flooded forests and tall dense macrophyte habitats during the wet season but not the dry season (Rejmankova et al. 1998). A discrepancy found in this study was the mean attempted human biting rate for *An. vestitipennis* was higher for the dry season than the wet season. This result is probably a reflection of the landing collection efforts by season in villages in Toledo. More than twice the number of

landing collections was conducted at each village in the dry, as opposed to the wet, season. As seen in this study, the seasonal pattern of increased malaria, during the wet season, supports Grieco's findings, which incriminate *An. vestitipennis* as an important vector in malaria transmission in Toledo District.

Of all the districts in Belize, Toledo District has had the most malaria infections due to *P. vivax.* Toledo district had higher adjusted incidence for *P. vivax* of all six districts (47 to 159 cases per 1000 population during the 10-year period) (Table 6). Comparatively, Toledo District, on average during 1989 through 1999, had a three-fold greater *P. vivax* incidence than Stann Creek District, the district with the second highest incidence, and a 25-fold greater incidence than Belize District, which had the lowest average incidence during the 10-year span.

There are compelling reasons that can account for the disparity in *P. vivax* burden between Toledo and the other districts. In addition to the ecological conditions in the district favoring the anthropophagic and endophilic vector species, the lifestyle of the people, as well as the social infrastructure, is the most primitive of the six districts. In the 1991 census, people in Toledo District, as opposed to any other district, reported the highest percentage of rivers/streams/creeks as their main source of drinking water, with the highest percentage of toilet facilities being either outhouses or no facilities whatsoever. The inhabitants of the district also reported the lowest use, nation-wide, of electricity (26%), and the highest use of kerosene (73%) as a source of lighting. The most commonly used fuel for cooking was wood (69%), unlike other districts which used gas. Of the houses surveyed in Toledo District in 1991 by the CSO, 52 percent had dirt floors, 58 percent had thatched roofs, 95 percent had undivided houses, 41 percent had

one bedroom houses, and 57 percent had five or more people living in a house. These housing statistics differ from those of the other five districts in Belize. The housing construction reported in the 1991 census has been cited in entomological surveys conducted in this district (Grieco et al. 2000, Roberts et al. 1993). The thatched roofing and opening in the walls constructed with sticks or wooden slats allow for more entry points for an endophagic vector like *An. vestitipennis.* Clearly, the lifestyle of a large number of Toledo's inhabitants places them in greater contact with vectors, which puts the population at a much greater risk for malaria.

On average during 1989 to 1999, *P. falciparum* cases in the Cayo district were highest at the end of the wet season while *P. vivax* cases increased with the onset of the wet season. This pattern held in 1997 for *P. falciparum* and in 1996 for *P. vivax.* However, in 1996, *P. falciparum* cases peaked in numbers in the early wet season, while in 1997, *P. vivax* cases were highest during the latter part of the wet season. An explanation for the inter-annual variation and seasonal pattern of malaria cases may be that all three vector species play important roles in malaria transmission in this district. The MOH vector surveys conducted in 1996 and 1997 commonly found *An. albimanus* in high numbers (ISA=1.2) at most sites followed by *An. darlingi* (ISA=3.6) and *An. vestitipennis* (ISA=4.8). The ELISA assay by Achee et. al. for CSP in mosquitoes collected in Cayo District reported three outdoor pools of *An. albimanus* infected with *P. vivax* variant 247, two pools of indoor pools of *An. vestitipennis* infected with *P. falciparum,* and one indoor pool of *An. darlingi* positive for *P. falciparum* (Achee et al. 2000). All the positive mosquitoes were from human landing collections during 1994

through 1997. Other entomologic surveys conducted in Cayo District have found *An. darlingi* larvae, or adults, along rivers and streams, and *An. albimanus* in similar habitats and in non-river water bodies (Manguin et al. 1996, Roberts et al. 1996). Together, the studies incriminate all three species as vectors in this district. The role played by the three vector species in malaria transmission in this district most likely depends on the environmental conditions that favor their breeding sites, and therefore their abundance during the year, as well as preventive measures used by the malaria control program, such as DDT indoor spraying. Timely, and consistent, residual house spraying by the malaria control programs may reduce *Anopheles* populations and malaria significantly.

The seasonal pattern of malaria cases in Corozal and Orange Walk Districts were generally similar. *Plasmodium falciparum* cases occurred in greater numbers either at the end of the wet season or in the dry season. A majority of *P. vivax* cases occurred in November in Corozal, and in January in both Orange Walk and Corozal. In entomological surveys in the two districts, larvae and adults of *An. albimanus, An. vestitipennis* and *An. darlingi* were collected. However, *An. darlingi* seem to occur in very few localities in both districts. *Anopheles vestitipennis* collected in indoor human landing collections tested positive for *P. falciparum* in an ELISA assay testing for CSP (Achee 2000). Larvae of this vector were found within two kilometers of villages in Corozal and Orange Walk Districts in October and November of 1996; they were also associated with tall dense macrophytes in the area, especially after heavy rains (Rejmankova et al. 1998). Of the two common vectors found in the two districts, *An. vestitipennis* is the more competent vector because of its endophagic behavior and higher

susceptibility to malaria [*P. falciparum*] infection. Higher populations of this vector following heavy rains may make *An. vestitipennis* the more important vector of malaria and it may be largely responsible for malaria transmission after heavy rains in the two districts.

Pools of *An. albimanus* collected during 1994 and 1997 in human landing collections tested positive for *P. vivax.* The CSP-positive pools of *An. albimanus* were from peri-domiciliary collections in Orange Walk District and an indoor collection in Corozal District. *Anopheles albimanus* larvae have been collected in various marsh and river habitats and are associated with cyanobacterial mats (Manguin et al. 1996, Rejmankova et al. 1996). During the wet season, the floating cyanobacterial mats are less abundant; whereas, in the dry season they reform and increase in abundance. Increased availability of mats in the dry season greatly promotes population growth of *An. albimanus.* High densities of *An. albimanus* adults have been collected near rivers in the dry season of northern Belize, as compared to distances greater than one kilometer (Roberts et al. 2002). Collection of water in marshes after the wet season and increased abundance of cyanobacterial mats support *An. albimanus* breeding; they also help explain the upsurge in malaria cases during January and other months of the dry season in the northern districts.

In Belize, the total number of cases and the incidence of *P. vivax* were highest in the years 1993 through 1995, while for *P. falciparum* both cases and incidence were highest in the years 1994 through 1996. The rise in the total number of cases spanning the four years has been cited in country reports (PAHO). Malaria control in Belize has

changed approach in recent years. Control efforts began in 1957 with a countrywide DDT residual house spray program that lasted until 1989 (Bangs 1999). The National Malaria Control Program (NMCP) then switched to intermittent spraying in 1990 and 1991. Only areas with high malaria cases were sprayed as a result of pressures to reduce the use of DDT (Roberts et al. 2002). In 1993, the National Pesticide Control Board banned the use of DDT for malaria control. In 1996, following a substantial rise in the number of malaria cases, the NMCP initiated a stratified residual house spray program using DDT and deltamethrin along with case detection and treatment.

It has been shown, in a study conducted by Roberts et. al in 2002, that reduced residual spraying resulted in the increase of both *P. vivax* and *P. falciparum* infections. The authors developed an index called the minimum effective house spray rate (MEHSR), using a logistic regression model that explained the relationship between the numbers of malaria cases and houses sprayed. A MEHSR of 134.6 houses per 1000 population (using the nation-wide spraying approach) would prevent an increase in malaria cases. In effect since 1995, the stratified house spraying control method (focal spraying), which uses a combination of DDT and deltamethrin, results in an MEHSR of 104. This figure, however, is lower than the 134.6-sprayed houses per 1000 required to prevent an increase in malaria cases using a non-stratified approach to control. Therefore, during 1993 to 1995, when no residual spraying (nationwide or focal) occurred, the numbers of *P. falciparum* and *P. vivax* infections increased drastically (82 and 64 percent respectively) compared to 1990 when some spraying occurred.

Clearly, the cost-effectiveness of control operations can be improved by using specific criteria for targeting spray operations. Examination of NMCP records, during

1997 through 2001, indicates residual house spraying operations, resources permitting, were generally conducted twice a year, before and after the wet season. Further benefits may result from targeting control by risk factors for falciparum versus vivax malarias. For instance, in Stann Creek, residual spraying during January through March may prove more effective in reducing human vector contact than conducting spraying operations during the wet season.

In conclusion, Stann Creek District had the highest *P. falciparum* incidence during the study period. The highest *P. falciparum* incidence was among children aged four or less. The common finding of *An. darlingi* implicates this species as a primary vector of *P. falciparum* in Stann Creek District. During 1989 to 1999, Cayo, Stann Creek and Toledo Districts experienced a greater burden of malaria (excess risk of 61 to 92%) than Corozal, Orange Walk, and Toledo Districts. As seen by the results of the MOH vector surveys, *Anopheles* species varied by district. *Anopheles albimanus,* a comparatively less competent vector, was not only present but abundant in most villages in Cayo, Stann Creek, and Toledo Districts.

Anopheles albimanus is the most common *Anopheles* mosquito in all districts (except Stann Creek). *Anopheles darlingi* was the second most common vector in Cayo and Toledo Districts. The higher rainfall, abundant rivers and forests in southern Belize provide suitable breeding habitats for *An. darlingi* and *An. vestitipennis*, which are both competent vectors of falciparum malaria. The variations in malaria incidence in Belize probably are likely due to a multitude of factors, some of which are ecological conditions

favoring vector presence and abundance, malaria control strategies, occupational migration, and social characteristics.

Suggestions for future studies in Belize are 1) entomologic surveys for *An. darlingi* habitats in wet and dry seasons along river systems in Stann Creek District; 2) studies documenting the biting behavior of vectors in human landing collections throughout the evening and night in historically high *P. falciparum* incidence villages in Stann Creek District; 3) longitudinal observational studies investigating behavioral risk factors for malaria among younger, versus older, age groups in Stann Creek villages; and 4) comparative susceptibility studies of the three main vector species to indigenous *P. falciparum* and *P. vivax*.

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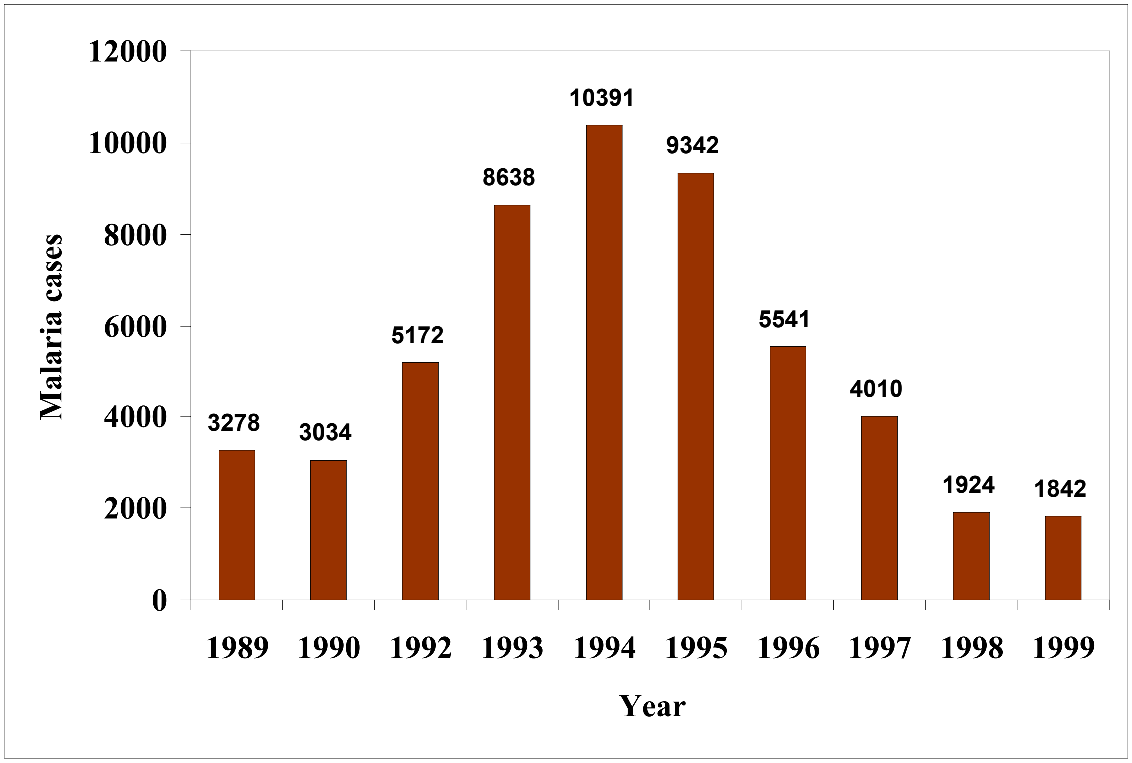


Figure 1: Total number of malaria cases in Belize during 1989 to 1999

Table 1
Distribution of *P. falciparum* and *P.vivax* cases by gender and by year

	Female		Male		Total	
	n	(%)	n	(%)	n	(% of all cases)
P. falciparum						
1989	30	(43)	40	(57)	70	(2)
1990	20	(44)	25	(56)	45	(1)
1992	68	(46)	81	(54)	155	(3)
1993	124	(49)	130	(51)	256	(3)
1994	218	(53)	193	(47)	411	(4)
1995	231	(49)	244	(51)	475	(5)
1996	179	(48)	194	(52)	374	(7)
1997	48	(39)	75	(61)	123	(3)
1998	70	(43)	94	(57)	164	(9)
1999	18	(39)	28	(61)	46	(3)
P. vivax						
1989	1309	(41)	1899	(59)	3208	(98)
1990	1314	(44)	1675	(56)	2989	(99)
1992	2130	(42)	2886	(58)	5017	(97)
1993	3557	(42)	4822	(58)	8382	(97)
1994	4354	(44)	5626	(56)	9980	(96)
1995	3794	(43)	5073	(57)	8867	(95)
1996	2248	(44)	2919	(56)	5167	(93)
1997	1707	(44)	2180	(56)	3887	(97)
1998	721	(41)	1039	(59)	1760	(91)
1999	813	(45)	983	(55)	1796	(97)

Table 2

Distribution of malaria cases by district, year, and species during 1989 to 1999†

	Corozal	**Orange Walk**	**Belize**	**Cayo**	**Stann Creek**	**Toledo**	**Country**
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n
P. falciparum							
1989	0 -	4 (12)	3 (9)	23 (68)	0 -	4 (12)	34
1990	0 -	0 -	2 (5)	34 (87)	2 (5)	1 (3)	39
1992	5 (4)	2 (2)	1 (1)	41 (33)	73 (58)	4 (3)	126
1993	17 (9)	3 (2)	5 (3)	101 (53)	43 (23)	20 (11)	189
1994	26 (9)	6 (2)	13 (5)	95 (33)	99 (35)	45 (16)	284
1995	7 (2)	6 (2)	13 (4)	157 (51)	99 (32)	25 (8)	307
1996	14 (5)	8 (3)	28 (9)	35 (11)	191 (63)	29 (10)	305
1997	3 (3)	16 (16)	21 (21)	36 (36)	13 (13)	10 (10)	99
1998	0 -	15 (11)	3 (2)	109 (82)	3 (2)	3 (2)	133
1999	1 (3)	7 (23)	1 (3)	13 (43)	7 (23)	1 (3)	30
P. vivax							
1989	352 (15)	392 (16)	126 (5)	794 (33)	207 (9)	508 (21)	2379
1990	413 (19)	223 (10)	101 (5)	736 (34)	117 (5)	606 (28)	2196
1992	441 (12)	677 (18)	166 (4)	1340 (36)	561 (15)	560 (15)	3745
1993	1015 (16)	854 (14)	349 (6)	1946 (31)	655 (11)	1370 (22)	6189
1994	1302 (18)	947 (13)	572 (8)	1950 (27)	875 (12)	1624 (22)	7270
1995	656 (10)	468 (7)	392 (6)	2208 (35)	1003 (16)	1666 (26)	6393
1996	264 (7)	138 (4)	159 (4)	1144 (31)	730 (20)	1298 (35)	3733
1997	177 (6)	100 (3)	108 (4)	795 (28)	477 (17)	1211 (42)	2868
1998	93 (7)	65 (5)	52 (4)	363 (28)	187 (15)	525 (41)	1285
1999	62 (5)	23 (2)	41 (3)	199 (16)	246 (20)	675 (54)	1246

† n = #(%) non-zero

Table 3
Distribution of 1991 census villages positive (having 1 or more case) for *P. vivax* and *P. falciparum* by district and by year[1]

	Corozal	**Orange Walk**	**Belize**	**Cayo**	**Stann Creek**	**Toledo**
	n=33(100%)	n=23(100%)	n=26(100%)	n=39(100%)	n=22(100%)	n=36(100%)
P.falciparum						
1989	0 -	3(13)	1(4)	11(28)	0 -	3(8)
1990	0 -	0 -	2(8)	9(23)	2(9)	1(3)
1992	5(15)	2(9)	1(4)	10(26)	8(36)	4(11)
1993	11(33)	2(9)	3(12)	22(56)	8(36)	9(25)
1994	15(45)	3(13)	3(12)	21(54)	10(45)	6(17)
1995	5(15)	2(9)	6(23)	29(74)	12(55)	8(22)
1996	6(18)	4(17)	10(38)	17(44)	14(64)	13(36)
1997	2(6)	7(30)	6(23)	16(41)	5(23)	7(19)
1998	0 -	4(17)	3(12)	19(49)	2(9)	3(8)
1999	1(3)	2(9)	1(4)	8(21)	3(14)	1(3)
P. vivax						
1989	31(94)	21(91)	12(46)	32(82)	16(73)	31(86)
1990	33(100)	21(91)	12(46)	36(92)	14(64)	33(92)
1992	29(88)	23(100)	14(54)	35(90)	17(77)	31(86)
1993	31(94)	23(100)	20(77)	37(95)	20(91)	33(92)
1994	30(91)	22(96)	20(77)	35(90)	19(86)	35(97)
1995	30(91)	21(91)	19(73)	35(90)	20(91)	34(94)
1996	28(85)	20(87)	18(69)	33(85)	22(100)	36(100)
1997	25(76)	19(83)	16(62)	35(90)	18(82)	34(94)
1998	22(67)	13(57)	8(31)	27(69)	19(86)	32(89)
1999	20(61)	10(43)	8(31)	31(79)	14(64)	35(97)

[1] 'N' indicates the number of villages in each district, as cited in the 1991 census of Belize. The numbers in parentheses are the proportion positive (or %) of the number of census villages in each district.

Table 4
Mean incidence per 1000 population of *P. vivax* and *P. falciparum* by district and by year

Year	Corozal	Orange Walk	Belize	Cayo	Stann Creek	Toledo
P. falciparum						
1989	0.0	0.1	0.1	0.6	0.0	0.4
1990	0.0	0.0	0.0	0.8	0.1	0.1
1992	0.2	0.1	0.0	1.0	4.8	0.4
1993	0.6	0.1	0.1	2.4	2.7	2.0
1994	1.0	0.2	0.2	2.3	6.2	4.5
1995	0.3	0.2	0.2	3.8	6.2	2.5
1996	0.5	0.3	0.5	0.8	11.7	2.9
1997	0.1	0.6	0.4	0.9	0.9	1.0
1998	0.0	0.7	0.1	2.7	0.2	0.3
1999	0.0	0.4	0.0	0.3	0.5	0.1
Average	0.3	0.3	0.2	1.6	3.3	1.4
P.vivax						
1989	12.6	13.9	2.4	19.3	13.8	54.1
1990	14.6	8.0	2.0	17.7	8.2	61.5
1992	15.8	23.0	3.3	32.3	36.7	57.9
1993	35.9	29.0	6.6	46.7	40.9	138.9
1994	48.5	32.3	10.8	47.0	54.9	161.5
1995	24.2	16.4	7.5	53.2	62.6	168.8
1996	10.0	5.1	3.1	27.7	44.8	128.3
1997	6.9	3.9	2.1	19.1	32.8	121.8
1998	4.0	2.8	1.1	9.1	11.8	54.6
1999	2.8	1.3	0.8	5.0	17.3	68.0
Average	17.5	13.6	4.0	27.7	32.4	101.5

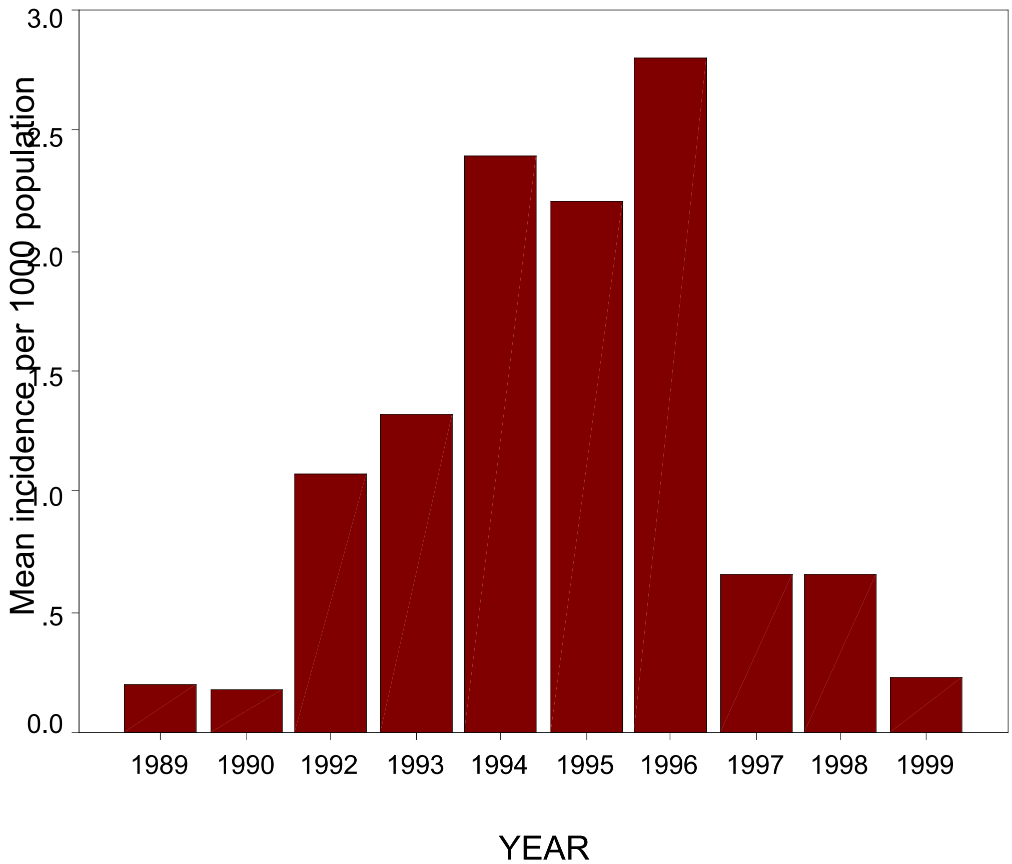


Figure 2: Plot of the mean *P. falciparum* incidence by year of study

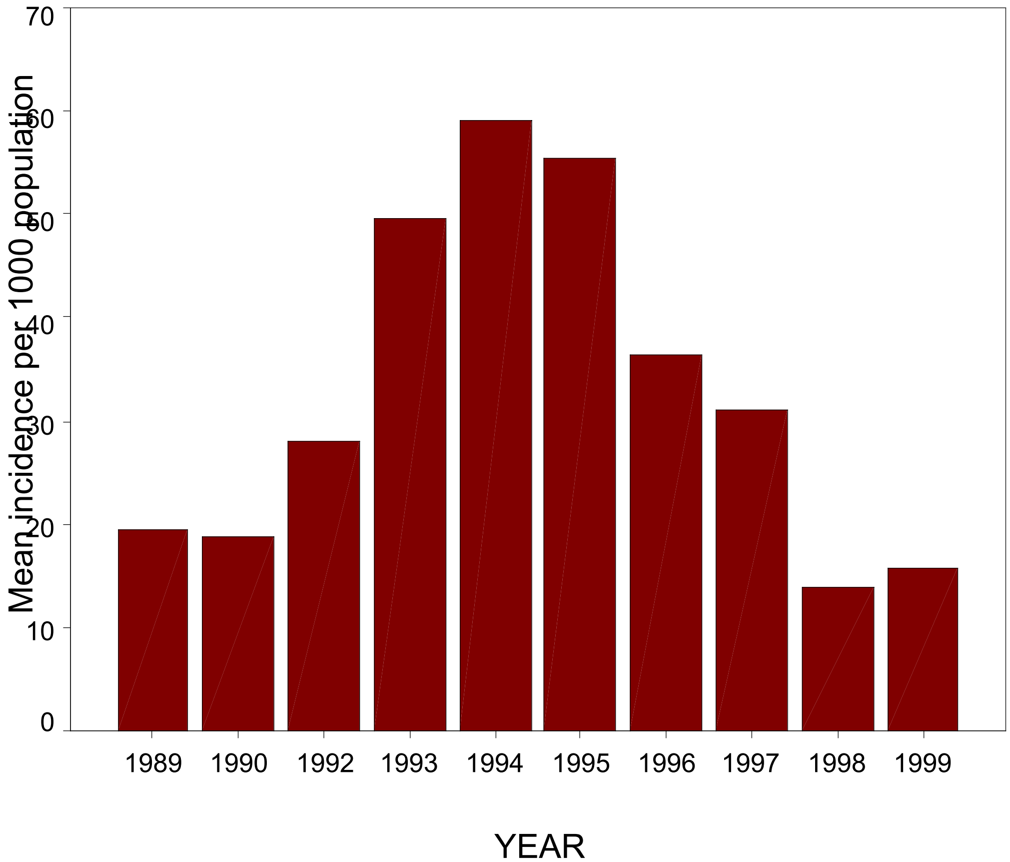


Figure 3: Plot of the mean *P. vivax* incidence by year of study

Table 5
Age and gender distribution of malaria, by species, incidence per 1000 population

	0 to 4	5 to 14	15 to 24	25 to 34	35 to 44	45 to 54	55 to 64	65 Plus
P. falciparum								
Female								
Corozal	0.3	0.2	0.2	0.3	0.8	0.2	0.3	0.3
OrangeWalk	0.2	0.2	0.1	0.7	0.3	0.4	0.0	0.3
Belize	0.1	0.2	0.2	0.1	0.4	0.4	0.4	0.4
Cayo	2.2	2.5	3.4	2.5	2.8	2.2	1.9	1.6
StannCreek	5.0	3.7	4.6	3.3	3.2	2.4	0.9	1.0
Toledo	0.7	1.5	1.1	1.7	1.5	2.7	1.0	0.8
Average	1.4	1.4	1.6	1.4	1.5	1.4	0.8	0.7
Male								
Corozal	0.0	0.2	0.2	0.4	0.5	0.2	0.7	0.4
OrangeWalk	0.1	0.3	0.1	0.4	0.2	0.5	0.0	0.2
Belize	0.0	0.2	0.2	0.3	0.5	0.2	0.4	0.4
Cayo	1.9	2.5	3.5	3.4	3.8	3.8	2.1	2.8
StannCreek	5.0	3.7	3.2	3.3	5.1	2.6	2.3	0.6
Toledo	1.1	1.4	1.3	1.0	1.8	1.3	1.8	1.3
Average	1.4	1.4	1.4	1.5	2.0	1.4	1.2	1.0
P. vivax								
Female								
Corozal	7.2	16.5	18.7	19.5	20	17.5	9.8	6.7
OrangeWalk	7	14.6	15.8	17	16.2	12.1	6.9	6.6
Belize	2.5	3.4	4.3	3	3.1	2.5	1.1	0.9
Cayo	39.9	49	45	44.3	41.7	29.9	20.8	15.6
StannCreek	38.9	41.9	38.5	29.6	30.4	17.9	11.8	6.7
Toledo	76.5	82.3	69.1	59.2	54.5	57.1	38.9	29.8
Male								
Corozal	6	12.7	25.1	23.2	22.1	23.7	10.1	8.1
OrangeWalk	6.8	13.9	20.7	17.8	16.5	17.2	8.2	6.5
Belize	2.2	3.8	8.3	7.2	6.2	4.1	1.8	1.3
Cayo	37.6	50.7	67.9	59.5	53.6	59.9	27.3	20.1
StannCreek	40.2	38.1	58.4	50.5	42.9	33.6	13.7	9.2
Toledo	78.4	81.6	77.3	66.5	69.5	53.7	34.6	30.6

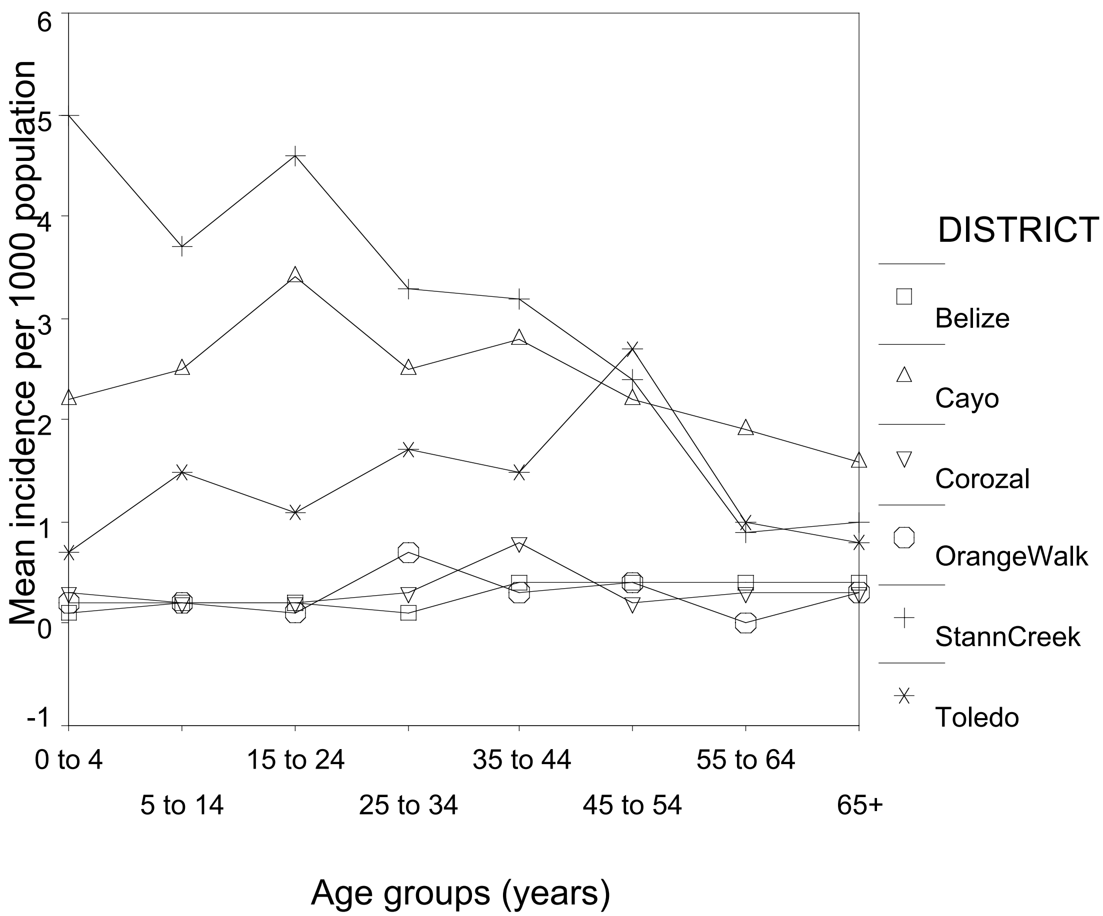


Figure 4a: *Plasmodium falciparum* incidence, by age group and district, among females

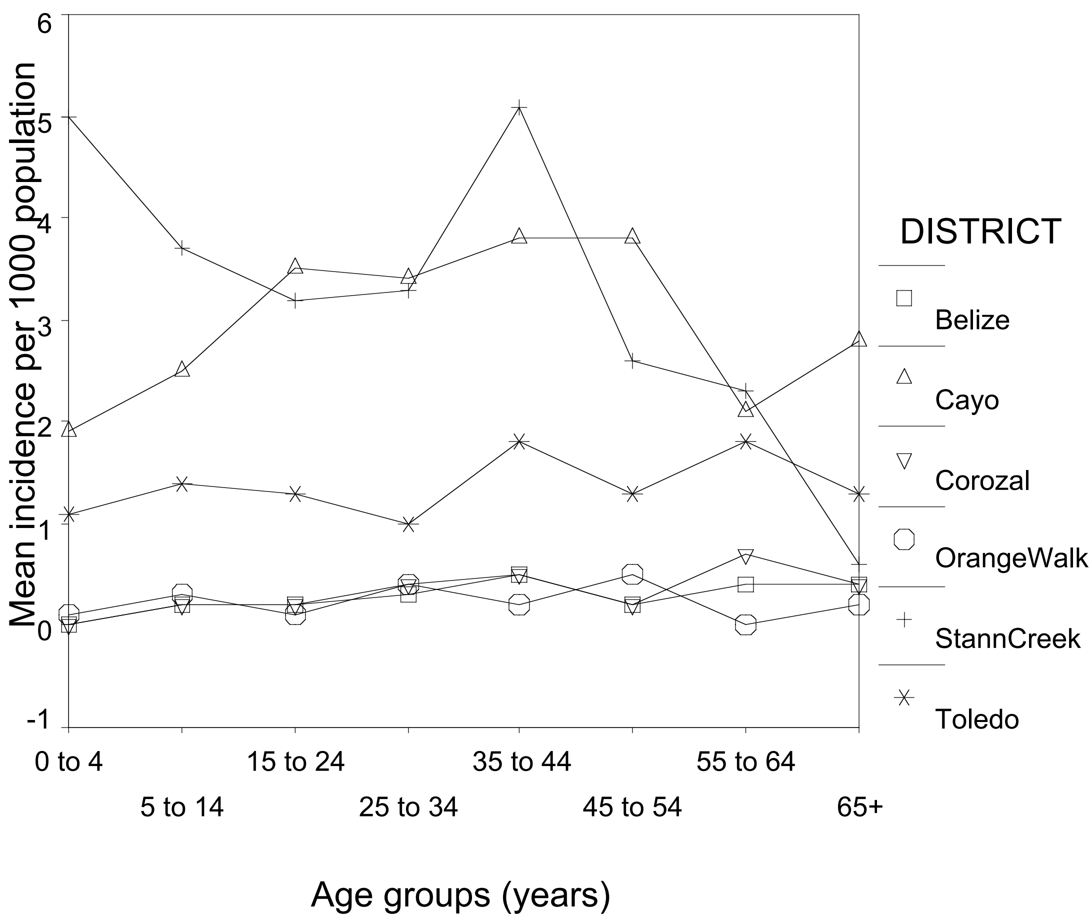


Figure 4b: *Plasmodium falciparum* incidence, by age group and district, among males

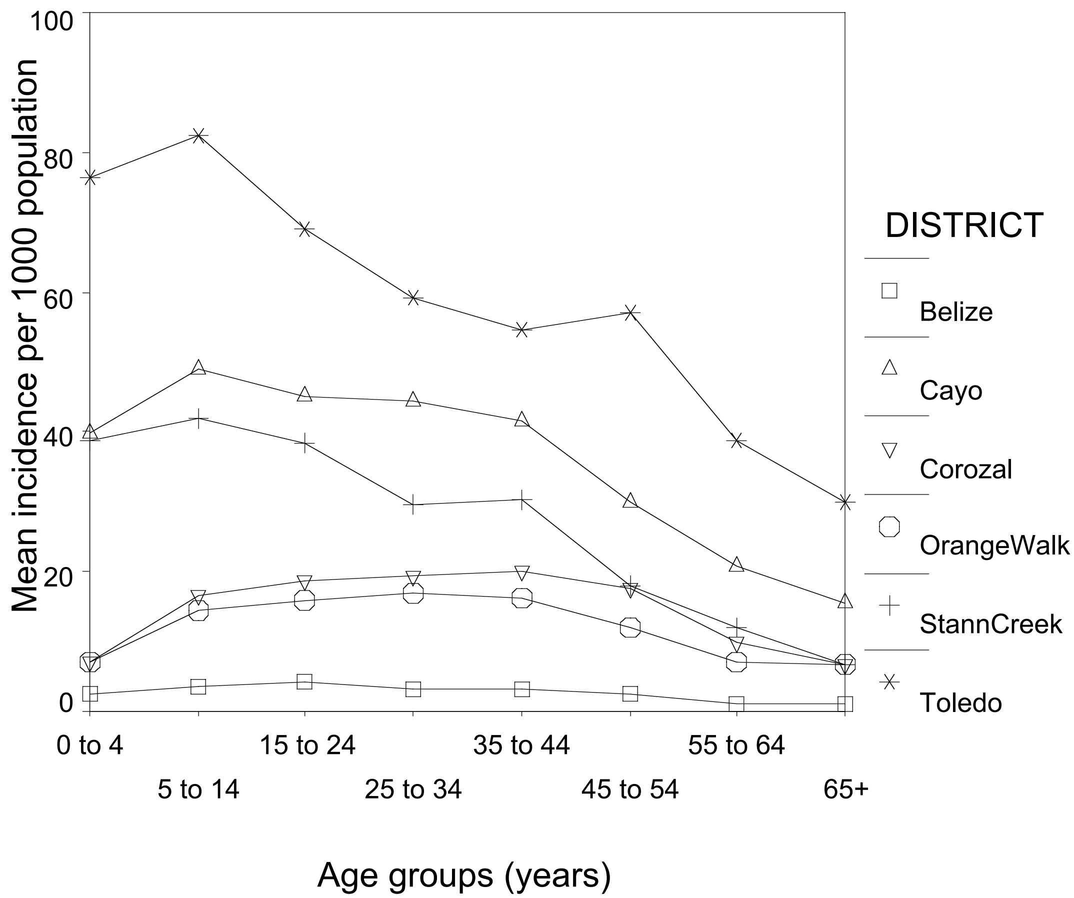


Figure 4c: *Plasmodium vivax* incidence, by age group and district, among females

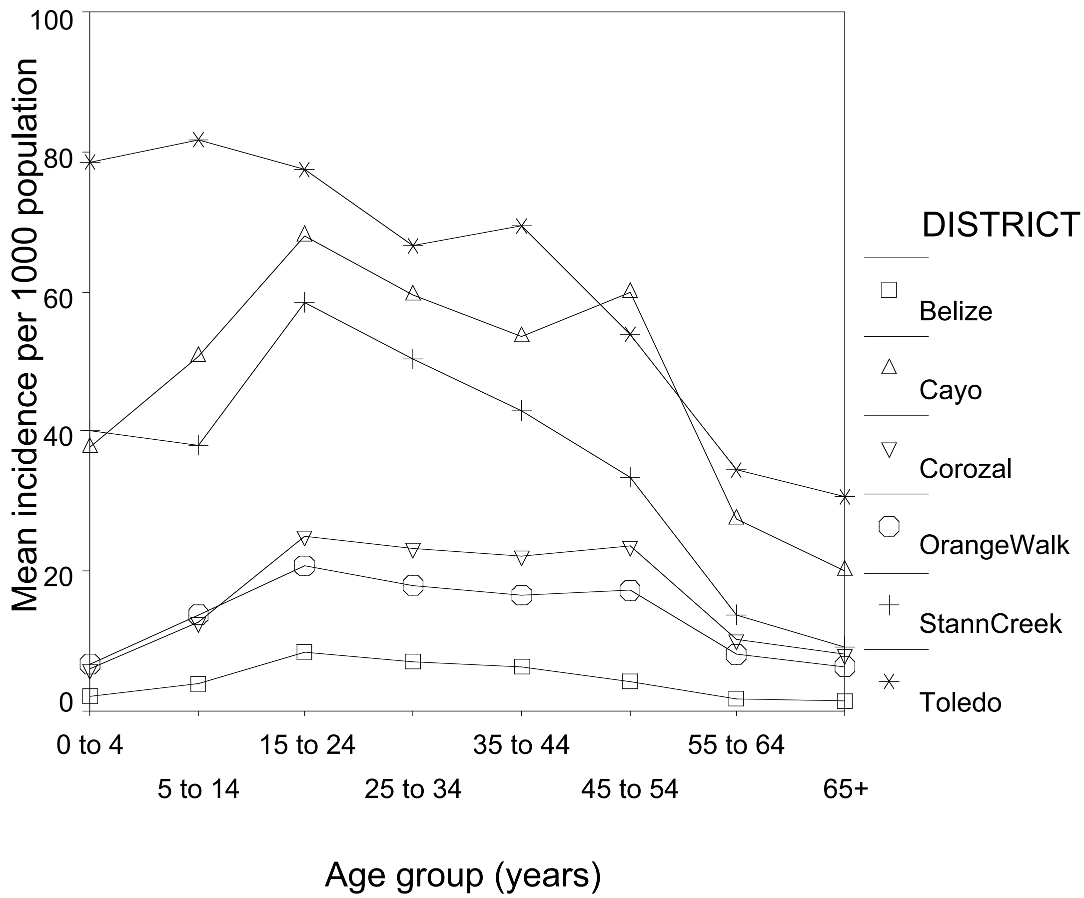


Figure 4d: *Plasmodium vivax* incidence, by age group and district, among males

Table 6
Adjusted incidence rate per 1000 population* of malaria, by species, for each district during 1989 to 1999

	1989	1990	1992	1993	1994	1995	1996	1997	1998	1999
*P. falciparum**										
Corozal	0.0	0.0	0.0	0.2	0.4	0.0	0.1	0.0	0.0	0.0
Orange Walk	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.1	0.0
Belize	0.0	0.0	0.0	0.0	0.0	0.1	0.2	0.1	0.0	0.0
Cayo	0.2	0.2	0.3	1.4	1.2	2.8	0.4	0.4	1.3	0.1
Stann Creek	0.0	0.0	1.7	1.0	2.8	3.4	7.5	0.2	0.0	0.1
Toledo	0.0	0.0	0.0	0.5	0.7	0.6	1.0	0.2	0.0	0.0
P. vivax@										
Corozal	11.8	14.6	13.9	33.8	44.1	22.0	8.5	5.2	2.6	1.7
Orange Walk	12.7	7.3	23.0	29.0	30.9	15.0	4.4	3.2	1.6	0.6
Belize	1.1	0.9	1.8	5.1	8.3	5.5	2.1	1.3	0.3	0.3
Cayo	15.8	16.3	28.9	44.3	42.1	47.7	23.5	17.2	6.3	4.0
Stann Creek	10.0	5.2	28.3	37.1	47.4	56.9	44.8	26.8	10.2	11.0
Toledo	46.6	56.4	49.9	127.4	157.0	159.4	128.3	115.0	48.6	66.1

*Adjusted incidence rate = $\frac{\text{(All. } P.falciparum\text{-positive villages)}}{\text{(All villages in the district)}} \times \frac{(P.falciparum \text{ cases})}{\text{(Population in } P.\ falciparum\text{-positive villages)}} \times 1000$

@ Adjusted incidence rate = $\frac{\text{(All. } P.\ vivax\text{-positive villages)}}{\text{(All villages in the district)}} \times \frac{(P.\ vivax \text{ cases})}{\text{(Population in } P.vivax\text{-positive villages)}} \times 1000$

Table 7

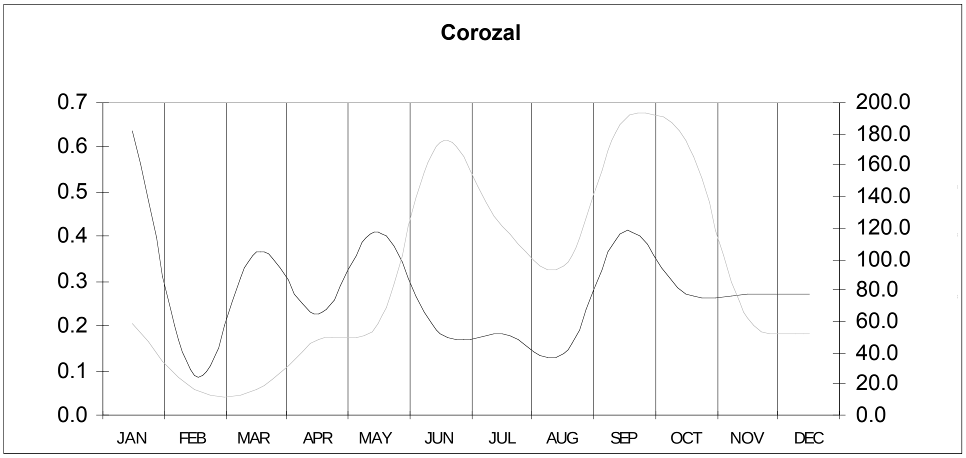
Mean incidence per 1000 population and attack rate percent (excess risk percent) for *P. vivax* and *P. falciparum* by region and by year

Region*	1989	1990	1992	1993	1994	1995	1996	1997	1998	1999
P. vivax (Mean incidence per 1000 population)										
South	23.0	22.2	37.0	58.8	65.9	72.3	46.9	37.6	16.5	17.6
North	8.1	6.9	11.8	20.1	25.9	14.0	5.3	3.7	2.2	1.4
Excess risk in the South (%)**	65	69	68	66	61	81	89	90	87	92
P. falciparum (Mean incidence per 1000 population)										
South	0.4	0.6	1.8	2.4	3.5	4.2	3.8	0.9	1.8	0.3
North	0.1	0	0.1	0.2	0.4	0.2	0.5	0.4	0.2	0.1
Excess risk in South (%)**	84	97	96	91	88	94	87	57	89	70

*North – Corozal, Orange Walk, and Belize Districts; South – Cayo, Stann Creek, and Toledo Districts

**Excess risk% = $\frac{(\text{South}_{\text{mean malaria incidence per 1000 population}} - \text{North}_{\text{mean malaria incidence per 1000 population}})}{\text{South}_{\text{mean malaria incidence per 1000 population}}} \times 100$

Figure 5: Average number of *P. falciparum* cases (black line) and average daily total precipitation (gray line) by month during 1989 through 1999 for all six districts in Belize.



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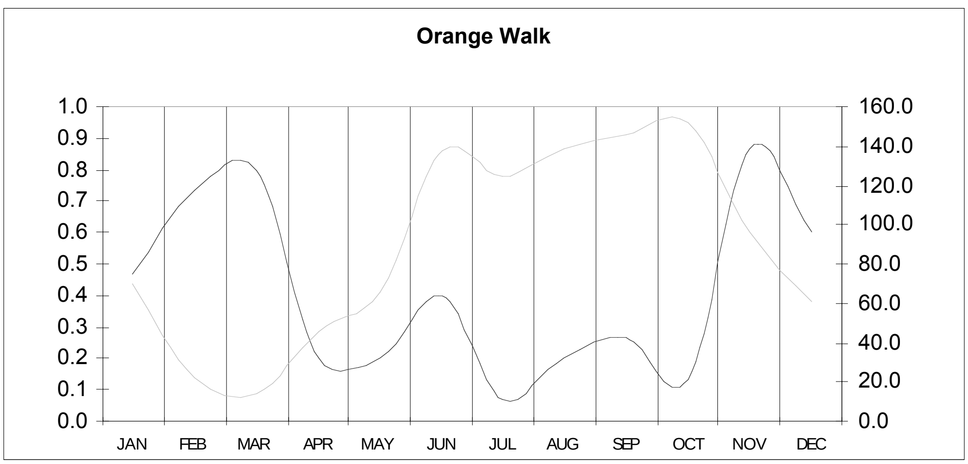

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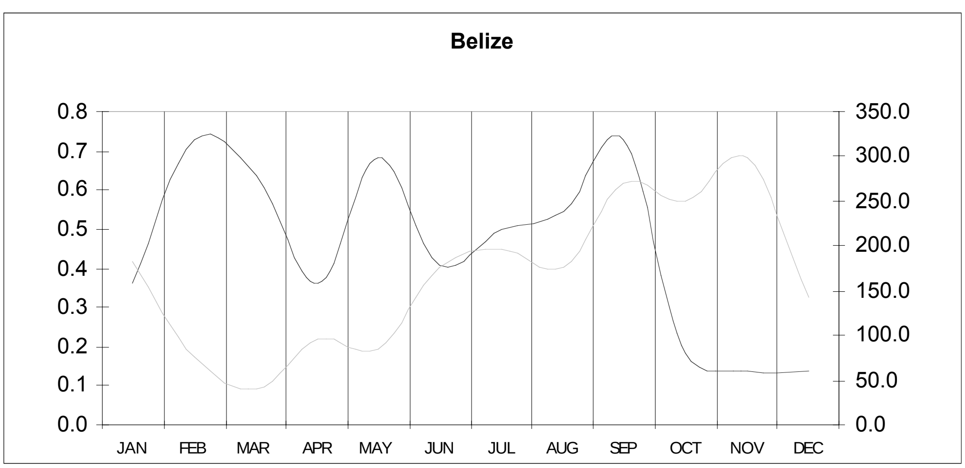

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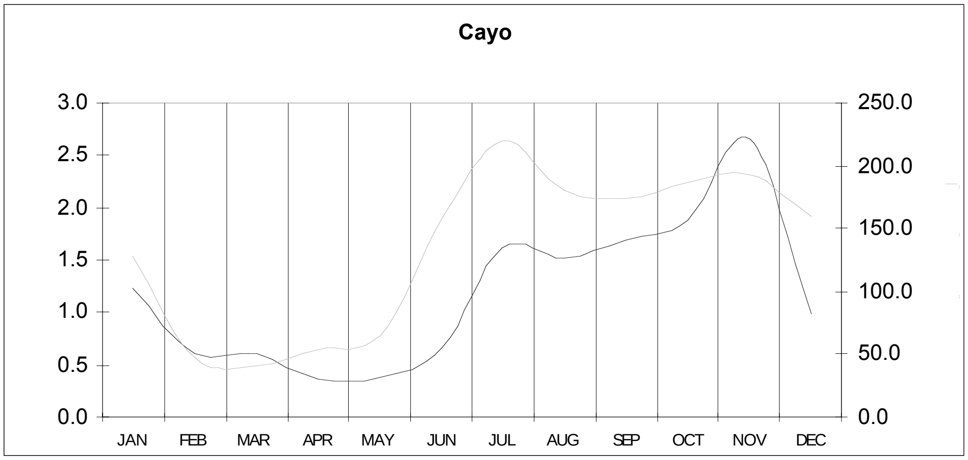
Figure 5 (cont.): Average number of *P. falciparum* cases (black line) and average daily total precipitation (gray line) by month during 1989 through 1999 for all six districts in Belize.



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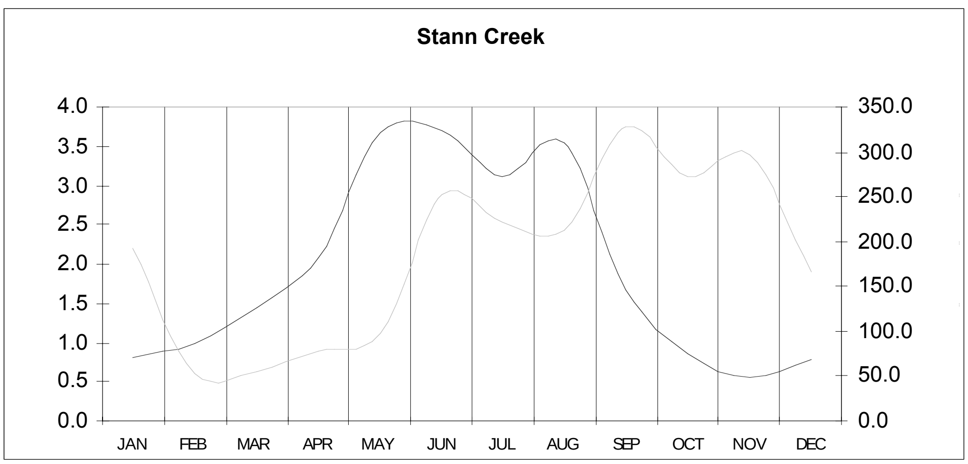

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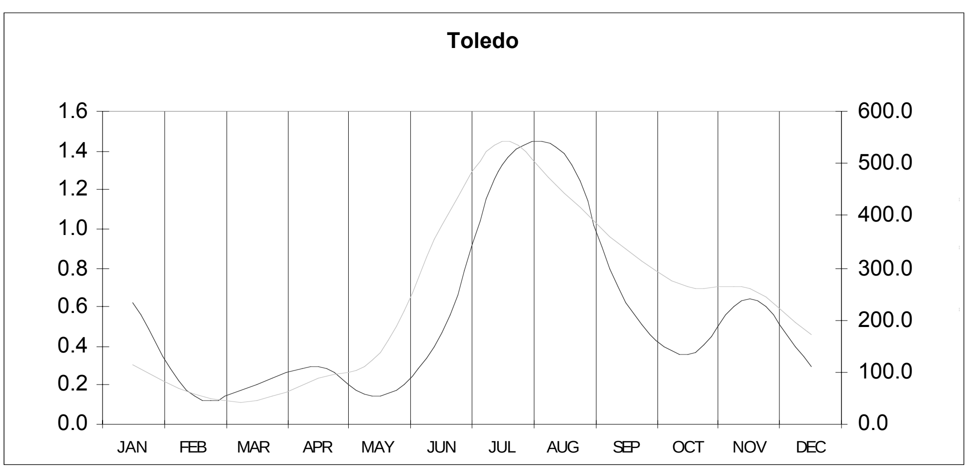

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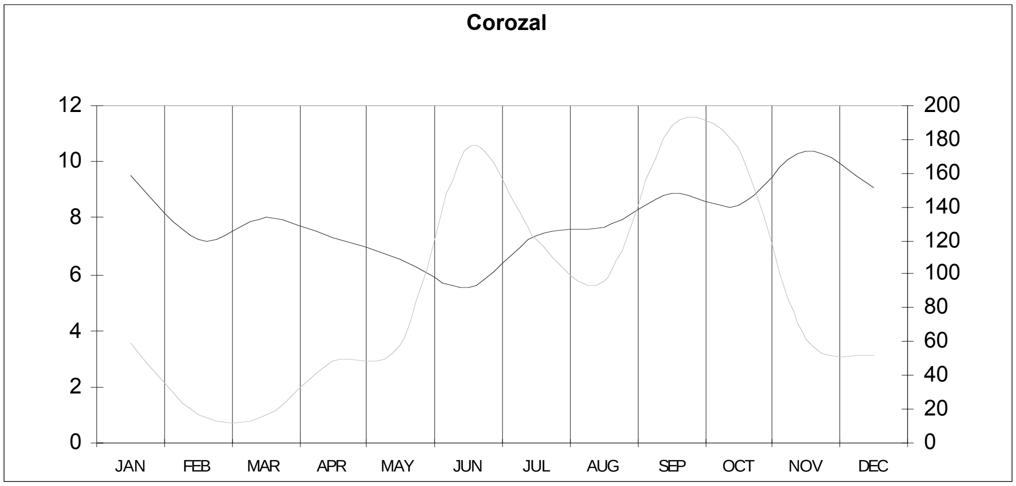
Figure 6: Average number of *P. vivax* cases (black line) and average daily total precipitation (gray line) by month during 1989 through 1999 for all six districts in Belize.



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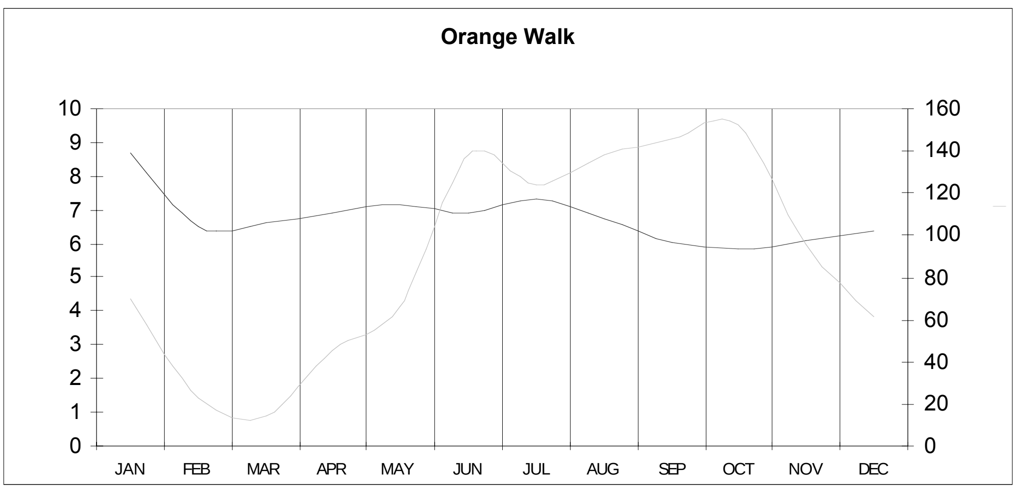

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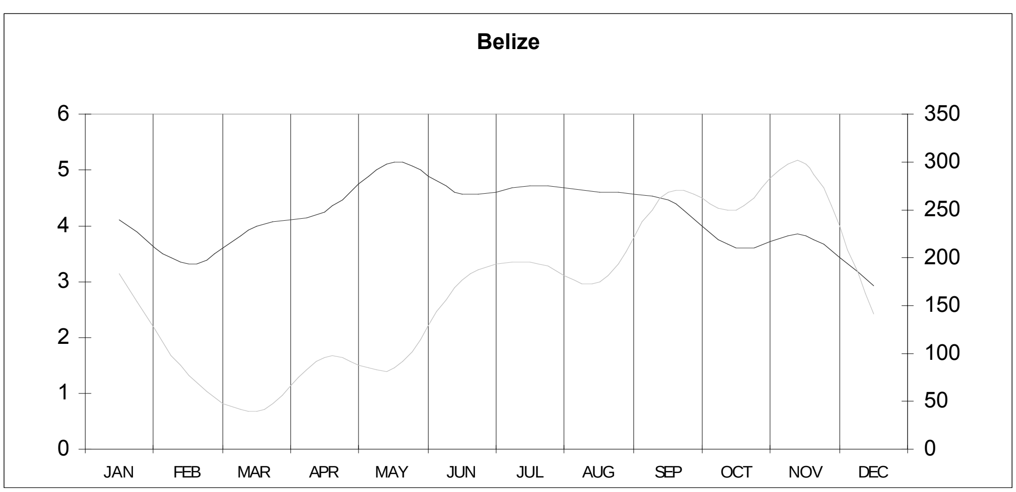
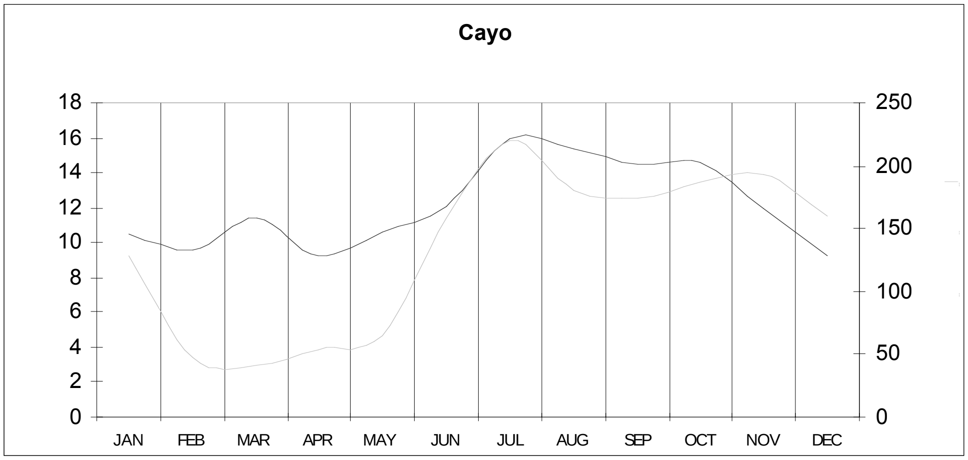


C

Figure 6 (cont.): Average number of *P. vivax* cases (black line) and average daily total precipitation (gray line) by month during 1989 through 1999 for all six districts in Belize.



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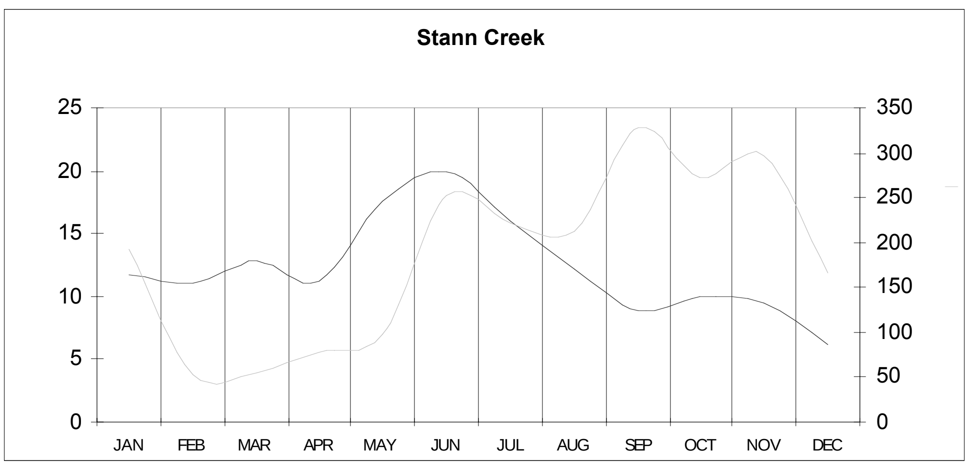

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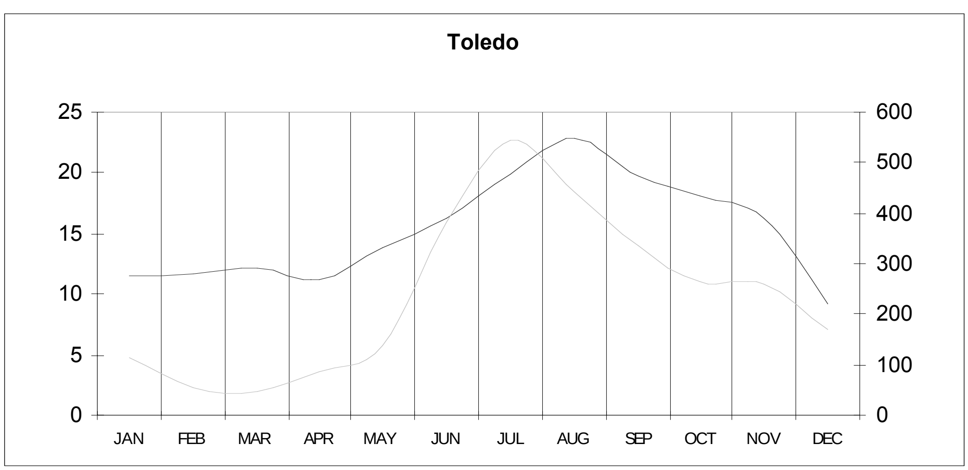

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Table 8
A list of the villages surveyed by the Ministry of Health during 1996 and 1997 in each district and the number of landing collections conducted in each village, by season

	Landing collections		
Cayo	Wet Season (Jul.-Dec.)	Dry Season (Jan.-Jun.)	Total
Billy White	8	2	10
Calla Creek	7	5	12
Camelote	5	5	10
Chial	6	4	10
Las Flores	2	0	2
Ontario	7	2	9
Roaring River	5	7	12
San Antonio	2	8	10
San Jose	2	0	2
San Martin	6	5	11
Unitedville	10	2	12
Valley of Peace	7	7	14
Stann Creek			
Alta Vista	7	4	11
Hope Creek	6	4	10
Pomona	4	8	12
Quarry	6	4	10
Red Bank	7	3	10
Riversdale	4	6	10
San Roman	7	5	12
Santa Rosa	10	2	12
Silk Grass	6	4	10
Sittee River	8	2	10
Toledo			
Big Falls	2	4	6
Columbia	4	2	6
Golden Stream	0	6	6
Indian Creek	0	6	6
Jacinto	2	4	6
Laguna	2	4	6
Rancho	2	4	6
San Antonio	2	4	6
San Jose	2	4	6
Santa Cruz	2	4	6

Table 9
Number of positive landing collections, by district, for three potential vectors during Aug. 21, 1996 through Nov. 14, 1997

	An. albimanus		*An. darlingi*		*An. vestitipennis*		Total no. of landing collections*
District	n	(%)	n	(%)	n	(%)	n
Cayo	65	(57)	26	(23)	11	(10)	114
Stann Creek	38	(36)	47	(44)	21	(20)	107
Toledo	18	(30)	6	(10)	3	(5)	60

* Landing collections were two hours (1800 to 2000 hours) in duration

Table 10

Number of *Anopheles* collected at villages in Cayo, Toledo and Stann Creek Districts during Aug. 1996 through Nov. 1997

Cayo	**Billy White**	**Calla creek**	**Camelote**	**Chial**	**Las Flores**	**Ontario**	**Roaring River**	**San Antonio**
An. albimanus	44	12	19	80	8	16	66	5
An. apicimacula	1	0	0	0	0	0	0	0
An. argyritarsis	0	0	0	0	0	0	0	0
An. crucians	1	0	0	0	0	0	1	0
An.darlingi	6	4	0	8	0	6	264	0
An.gabaldoni	0	0	0	0	0	0	0	0
An. pseudopunctipennis	0	0	1	0	0	0	0	0
An. punctimacula	2	1	1	0	0	0	3	0
An. vestitipennis	0	1	0	5	0	1	4	0
Stann Creek	**Alta Vista**	**Hope Creek**	**Pomona**	**Quarry**	**Red Bank**	**Riversdale**	**San Roman**	**Santa Rosa**
An. albimanus	2	10	**3**	14	8	0	27	21
An. apicimacula	0	0	0	0	0	0	0	1
An. argyritarsis	0	0	0	0	0	0	0	0
An. crucians	0	0	0	0	0	0	0	0
An.darlingi	5	16	18	96	53	4	119	61
An.gabaldoni	0	1	0	0	0	0	0	3
An. pseudopunctipennis	0	1	0	1	0	0	0	4
An. punctimacula	0	3	0	3	1	0	1	10
An. vestitipennis	0	5	1	4	1	0	13	20
Toledo	**Big Falls**	**Columbia**	**Golden Stream**	**Indian Creek**	**Jacinto**	**Laguna**	**Rancho**	**San Antonio**
An. albimanus	3	2	5	1	2	0	3	0
An. apicimacula	0	0	0	0	0	0	0	0
An. argyritarsis	0	0	0	0	0	0	0	0
An. crucians	0	0	0	0	0	0	0	0
An.darlingi	4	2	11	11	3	0	8	2

An.gabaldoni	0	0	0	0	0	0	0	0
An. pseudopunctipennis	0	0	0	0	0	0	0	0
An. punctimacula	0	0	2	0	0	0	0	0
An. vestitipennis	0	1	2	0	1	0	0	0

Table 10 (cont.)
Number of *Anopheles* collected at villages in Cayo, Toledo and Stann Creek Districts during Aug. 1996 through Nov. 1997

Cayo	**San Jose**	**San Martin**	**Unitedville**	**Valley of Peace**	**TOTAL**
An. albimanus	6	36	23	49	364
An. apicimacula	0	0	2	0	3
An. argyritarsis	0	0	0	0	0
An. crucians	0	0	0	0	2
An. darlingi	0	1	27	0	316
An. gabaldoni	0	0	3	1	4
An. pseudopunctipennis	0	0	0	0	1
An. punctimacula	0	3	0	0	10
An. vestitipennis	0	2	0	1	14
Stann Creek	**Silk Grass**	**Sittee River**			
An. albimanus	7	7			99
An. apicimacula	0	0			1
An. argyritarsis	0	0			0
An. crucians	0	0			0
An. darlingi	61	7			440
An. gabaldoni	0	0			4
An. pseudopunctipennis	0	1			7
An. punctimacula	0	3			21
An. vestitipennis	2	5			51
Toledo	**San Jose**	**Santa Cruz**			
An. albimanus	0	1			17
An. apicimacula	0	0			0
An. argyritarsis	0	0			0
An. crucians	0	0			0

An. darlingi	1	5	47
An. gabaldoni	0	0	0
An. pseudopunctipennis	0	0	0
An. punctimacula	0	0	2
An. vestitipennis	0	0	4

Table 11

The number of *Anopheles*, adjusted to the number of landing collections per village, collected in three districts

Cayo	**Billy White**	**Calla Creek**	**Camalote**	**Chial**	**Las Flores**	**Ontario**	**Roaring River**	**San Antonio**
An. albimanus	5.9	1.8	3.8	13.5	4.0	2.3	11.5	0.6
An. apicimacula	0.1							
An. argyritarsis								
An. crucians	0.1						0.2	
An. darlingi	0.6	0.4		1.3		0.9	44.6	
An. gabaldoni								
An. pseudopunctipennis			0.2					
An. punctimacula	0.3	0.2	0.2				0.4	
An. vestitipennis				0.8		0.1	0.7	
Stann Creek	**Alta Vista**	**Hope Creek**	**Pomona**	**Quarry**	**Red Bank**	**Riversdale**	**San Roman**	**Santa Rosa**
An. albimanus	0.4	2.8	0.4	0.7	1.3		4.7	0.8
An. apicimacula		0.8		0.5	0.1			2.1
An. argyritarsis								
An. crucians		0.2			0.1	0.9		0.3
An. darlingi	1.1	0.6	3.0	20.4	9.7		21.3	14.9
An. gabaldoni		0.2						0.1
An. pseudopunctipennis								
An. punctimacula				0.2			0.2	2.0
An. vestitipennis		1.7	0.3	2.3			2.0	1.0
Toledo	**Big Falls**	**Columbia**	**Golden Stream**	**Indian Creek**	**Jacinto**	**Laguna**	**Rancho**	**San Antonio**
An. albimanus	1.0	0.5	1.8	1.8	0.8		3.5	1.0
An. apicimacula					0.5			
An. argyritarsis								
An. crucians								
An. darlingi	1.0		0.3	0.2			0.8	
An. gabaldoni								
An. pseudopunctipennis								
An. punctimacula		0.5	0.8					
An. vestitipennis		0.3	0.3		0.5			

Table 11(cont.)
The number of *Anopheles*, adjusted to the number of landing collections per village, collected in three districts

Cayo	**San Jose**	**San Martin**	**Unitedville**	**Valley of Peace**
An. albimanus	3.0	5.9	2.9	7.0
An. apicimacula			0.2	
An. argyritarsis				
An. crucians				
An. darlingi		0.2	3.0	
An. gabaldoni			0.3	0.1
An. pseudopunctipennis				
An. punctimacula		0.5		
An. vestitipennis		0.2		0.1
Stann Creek	**Silk Grass**	**Sittee River**		
An. albimanus	14.5	0.6		
An. apicimacula		0.9		
An. argyritarsis				
An. crucians	1.2	0.4		
An. darlingi	0.5			
An. gabaldoni				
An. pseudopunctipennis				
An. punctimacula		0.1		
An. vestitipennis		0.9		
Toledo	**San Jose**	**Santa Cruz**		
An. albimanus	0.5	2.5		
An. apicimacula				
An. argyritarsis				
An. crucians				
An. darlingi				
An. gabaldoni		0.5		
An. pseudopunctipennis				
An. punctimacula				
An. vestitipennis				

Table 12

Ranks assigned to *Anopheles* collected at each village in three districts, and the *a*, *c*, Rj_j, and ISA for each vector species*

Cayo	**Billy White**	**Calla Creek**	**Camalote**	**Chial**	**Las Flores**	**Ontario**	**Roaring River**	**San Antonio**
An. albimanus	1.0	1.0	1.0	1.0	1.0	1.0	2.0	1.0
An. apicimacula	3.5							
An. argyritarsis								
An. crucians	3.5						5.0	
An. darlingi	2.0	2.0		2.0		2.0	1.0	
An. gabaldoni								
An. pseudopunctipennis			2.5					
An. punctimacula	3.0	3.0	2.5				4.0	
An. vestitipennis				3.0		3.0	3.0	
Stann Creek	**Alta Vista**	**Hope Creek**	**Pomona**	**Quarry**	**Red Bank**	**Riversdale**	**San Roman**	**Santa Rosa**
An. albimanus	2.0	1.0	2.0	3.0	2.0		2.0	5.0
An. apicimacula		3.0		4.0	3.5			2.0
An. argyritarsis								
An. crucians		5.5			3.5	1.0		6.0
An. darlingi	1.0	4.0	1.0	1.0	1.0		1.0	1.0
An. gabaldoni		5.5						7.0
An. pseudopunctipennis								
An. punctimacula				5.0			4.0	3.0
An. vestitipennis		2.0	3.0	2.0			3.0	4.0
Toledo	**Big Falls**	**Columbia**	**Golden Stream**	**Indian Creek**	**Jacinto**	**Laguna**	**Rancho**	**San Antonio**
An. albimanus	1.5	1.5	1.0	1.0	1.0		1.0	1.0
An. apicimacula					1.5			
An. argyritarsis								
An. crucians								
An. darlingi	1.5		3.5	2.0			2.0	
An. gabaldoni								
An. pseudopunctipennis								
An. punctimacula		1.5	2.0					
An. vestitipennis		2.0	3.5		1.5			

*ISA = $\frac{a + R_j}{K}$ R_j = sum of ranks in each rowa = (sum of zero cells in K columns) x (c) c = (largest rank in K columns + 1)

Table 12 (cont.)

Ranks assigned to *Anopheles* collected at each village in three districts, and the *a*, *c*, *Rj_j*, and ISA for each species*

Cayo	**San Jose**	**San Martin**	**Unitedville**	**Valley of Peace**	**Rj**	**a**	**c**	**ISA**
An. albimanus	1.0	1.0	2.0	1.0	14.0	0.0	6.0	1.2
An. apicimacula			4.0		7.5	60.0	6.0	5.6
An. argyritarsis					0.0	72.0	6.0	6.0
An. crucians					8.5	60.0	6.0	5.7
An. darlingi		3.5	1.0		13.5	30.0	6.0	3.6
An. gabaldoni			3.0	2.5	5.5	60.0	6.0	5.5
An. pseudopunctipennis					2.5	66.0	6.0	5.7
An. punctimacula		2.0			14.5	42.0	6.0	4.7
An. vestitipennis		3.5		2.5	15.0	42.0	6.0	4.8
Stann Creek	**Silk Grass**	**Sittee River**						
An. albimanus	1.0	2.0			20.0	8.0	8.0	2.8
An. apicimacula		1.5			14.0	32.0	8.0	4.6
An. argyritarsis					0.0	80.0	8.0	8.0
An. crucians	2.0	3.0			21.0	32.0	8.0	5.3
An. darlingi	3.0				13.0	16.0	8.0	2.9
An. gabaldoni					12.5	64.0	8.0	7.7
An. pseudopunctipennis					0.0	80.0	8.0	8.0
An. punctimacula		4.0			16.0	48.0	8.0	6.4
An. vestitipennis		1.5			15.5	32.0	8.0	4.8
Toledo	**San Jose**	**Santa Cruz**						
An. albimanus	1.0	1.0			10.0	4.5	4.5	1.5
An. apicimacula					1.5	40.5	4.5	4.2
An. argyritarsis					0.0	45.0	4.5	4.5
An. crucians					0.0	45.0	4.5	4.5
An. darlingi					9.0	27.0	4.5	3.6
An. gabaldoni		2.0			2.0	40.5	4.5	4.3
An. pseudopunctipennis					0.0	45.0	4.5	4.5
An. punctimacula					3.5	36.0	4.5	4.0
An. vestitipennis					7.0	31.5	4.5	3.9

*ISA = $\frac{a + R_j}{K}$ R_j = sum of ranks in each rowa = (sum of zero cells in K columns) x (c) c = (largest rank in K columns + 1)

Table 13

ISA, adjusted across seasons by district, for indoor and outdoor collections in 1996 and 1997

Cayo	ISA(Outdoor)	ISA(Indoor)
An. albimanus	1.1	2.3
An. apicimacula	5.5	5.0
An. argyritarsis	5.5	5.0
An. crucians	5.3	5.0
An. darlingi	3.6	3.9
An. gabaldoni	5.2	5.0
An. pseudopunctipennis	5.3	5.0
An. punctimacula	4.3	4.9
An. vestitipennis	4.8	4.3
Stann Creek		
An. albimanus	3.5	2.6
An. apicimacula	5.0	3.2
An. argyritarsis	7.0	3.3
An. crucians	5.0	3.1
An. darlingi	2.7	2.3
An. gabaldoni	7.0	3.1
An. pseudopunctipennis	7.0	3.3
An. punctimacula	5.8	3.2
An. vestitipennis	4.4	2.5
Toledo		
An. albimanus	2.0	1.8
An. apicimacula	4.6	3.0
An. argyritarsis	5.0	3.0
An. crucians	5.0	3.0
An. darlingi	3.9	2.9
An. gabaldoni	4.7	3.0
An. pseudopunctipennis	5.0	3.0
An. punctimacula	4.3	3.0
An. vestitipennis	4.2	3.0

Table 14
Mean human biting rate,* by district and by season, for three potential vectors

	Wet season (Jul. – Dec.)	Dry season (Jan. – Jun.)
An. albimanus		
Cayo	1.8	0.7
Stann Creek	0.5	0.8
Toledo	0.5	0.4
An. darlingi		
Cayo	1.2	0.6
Stann Creek	1.2	1.9
Toledo	0	0.1
An. vestitipennis		
Cayo	0.1	0.04
Stann Creek	0.4	0.02
Toledo	0.03	0.1

*Human biting rate = No. of *Anopheles*/No. of collectors per site/two-hour landing collection

Figure 7: Average *P. falciparum* cases (grey bar) and average daily total precipitation (grey line) by month in 1996 and 1997. Malaria cases are averaged for the villages in Cayo, Stann Creek, and Toledo Districts where vector surveys were conducted.

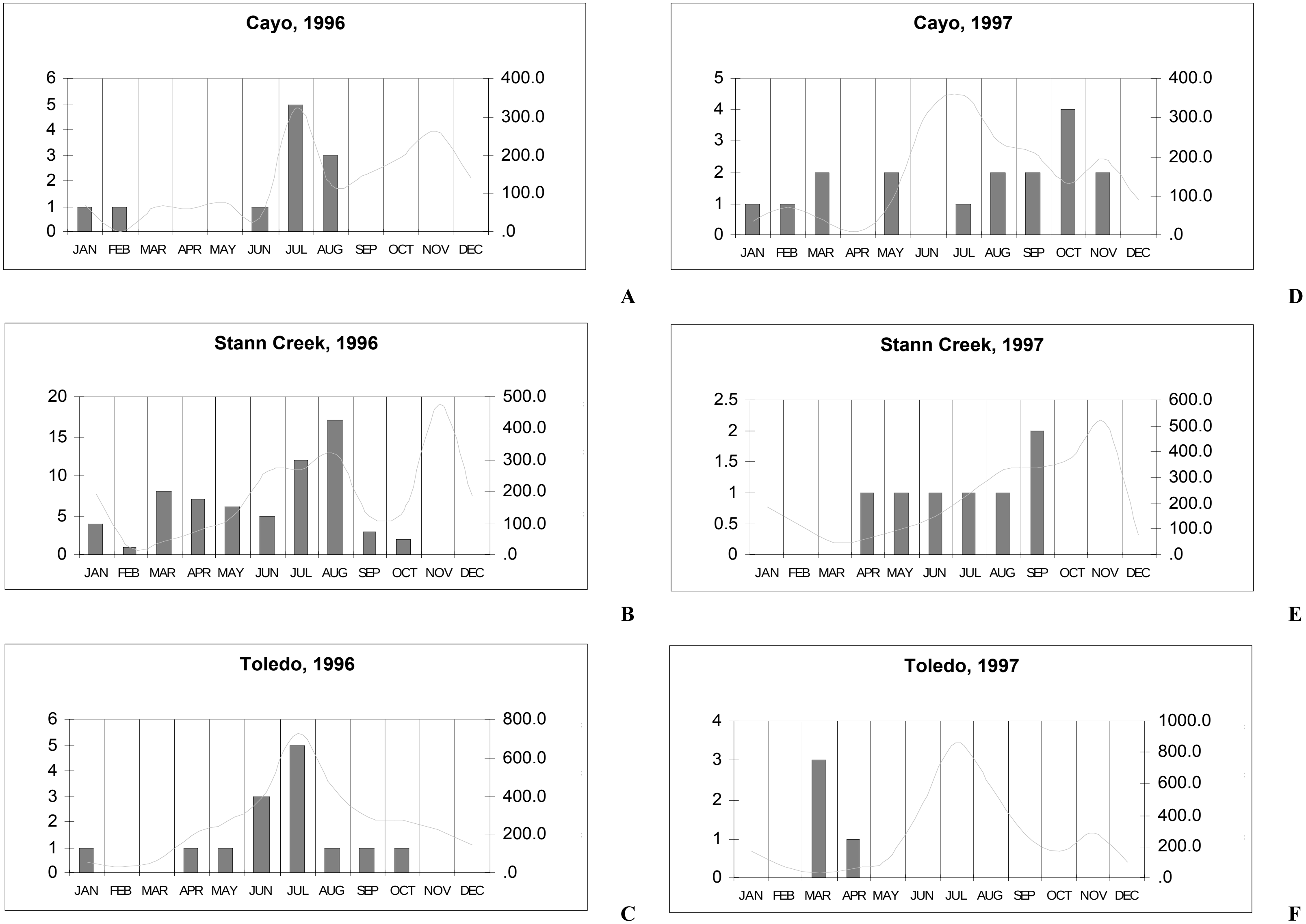
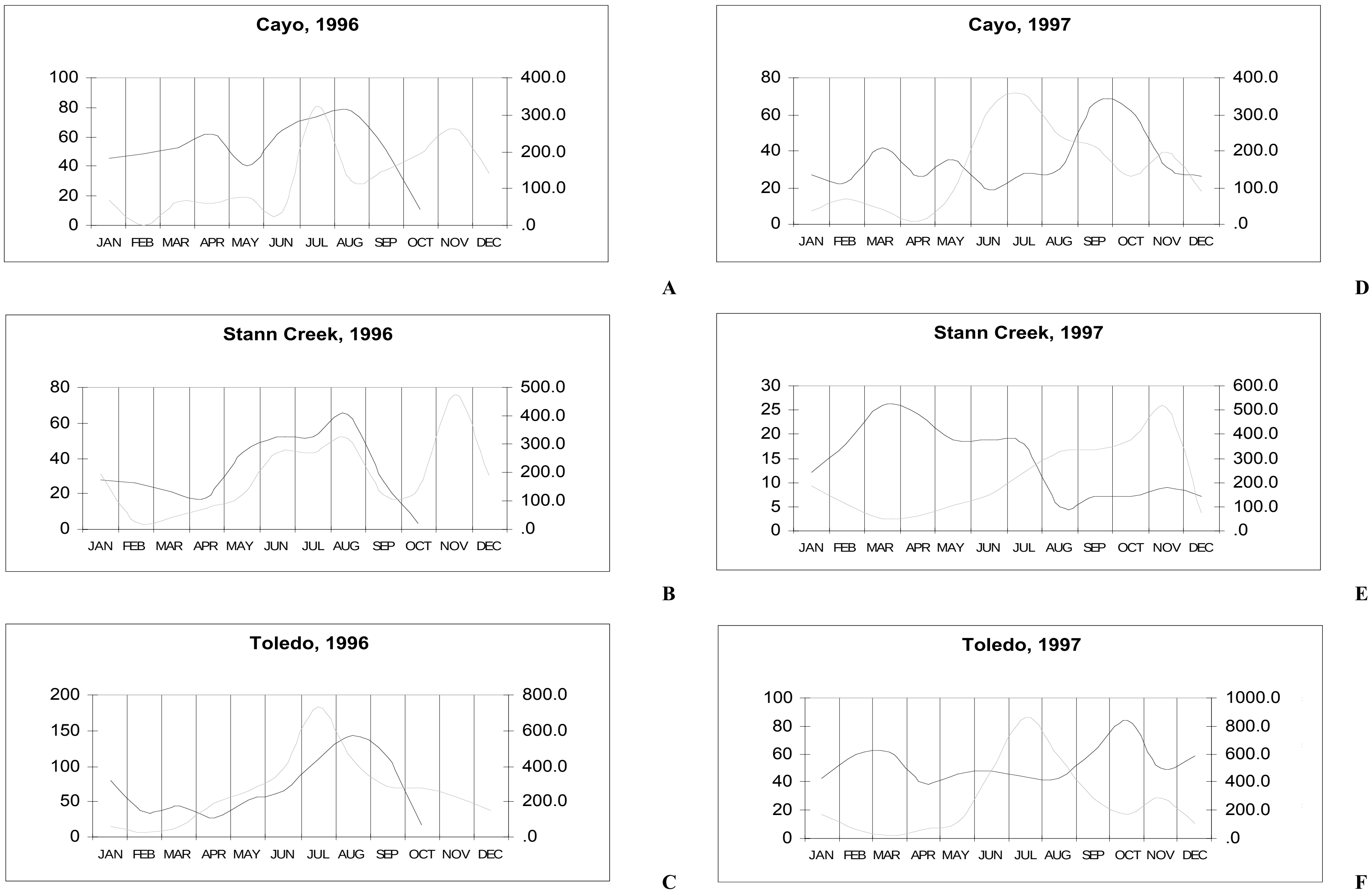


Figure 8: Average *P. vivax* cases (black line) and average daily total precipitation (grey line) by month in 1996 and 1997. Malaria cases are averaged for the villages in Cayo, Stann Creek, and Toledo Districts where vector surveys were conducted.



CHAPTER 4

Manuscript 3

Spatial statistical analysis of environmental risk factors for malaria in Belize, Central America

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ABSTRACT

The epidemiology of vector-borne diseases is directly influenced by vector characteristics, which are closely linked to environmental conditions. Malaria transmission results from complex interactions among humans, vectors, and the malaria parasite. The objective of this retrospective study was to determine if environmental factors such as land cover, topography, distance to rivers and streams, and weather data were associated with malaria incidence in villages.

Malaria information for 1993-1995, for 179 villages, was compiled from an electronic database maintained by the Belize National Malaria Control Program. The 1991 population census was used to calculate malaria incidence rates during 1993 – 1995. The geographic locations of villages in this study, elevation, slope, distance of villages to rivers and the coast were obtained from a digital data set. Weather data for the villages were obtained from satellite data and adjusted to ground weather station data. Land

cover in study villages was assessed by field-checking land cover classes obtained from a Landsat image. Univariate, bivariate and multivariate analyses were conducted to determine risk factors for malaria incidence in a village.

Higher averaged monthly daily total precipitation, higher averaged monthly minimum temperature, higher elevation, and greater percent forest cover were individually associated with malaria incidence ($p<0.05$). Multivariate analyses using a Poisson regression model that included all 179 villages indicated an increase in precipitation by 10 millimeters (RR=1.008; 95% C.L.= 1.006,1.01 $p<0.0001$) and an increase in forest cover by 10 percent, (RR=2.16; 95% C.L.= 2.08, 2.24 $p<0.0001$) were associated with higher malaria risk in a village. Adjusting the model for smaller ecological areas resulted in significantly different results for regions. Increased precipitation was significantly associated with higher malaria risk in villages in Cayo and Toledo. However, in the northern districts of Corozal and Orange Walk, decreased precipitation was associated with higher malaria risk in villages. This finding may be explained by the dry season abundance of *An. albimanus,* possibly the main vector in the two districts. Higher percentage of forest was significantly associated with higher malaria risk in Belize and Stann Creek Districts.

KEYWORDS: Malaria, GIS, remote sensing, environment, vectors, Belize

INTRODUCTION

The epidemiology of vector-borne diseases is directly influenced by vector characteristics, which are closely linked to environmental conditions. Malaria transmission results from complex interactions among humans, vectors, and the malaria parasite. In areas of high and low malaria transmission, variations have been noted within and/or among villages in Papua New Guinea, Liberia, Mozambique, Sri Lanka, and Pakistan (Bjorkman et al. 1985, Cattani et al. 1986, Gamage-Mendis et al. 1991, Hii et al. 1997, Strickland et al. 1987, Thompson et al. 1997, van der Hoek et al. 1998). Transmission variation may be associated with many factors, for example landscape variables, vector ecology, and human population density (Barrera et al. 1999). An understanding of the factors influencing the variation in transmission patterns within localities and regions will help in developing an effective malaria control program.

Spatial and temporal variations in humidity, rainfall and temperature influence the duration of the parasite's extrinsic incubation period, vector distribution, vector density and vector survival (Onori et al. 1980). Study of these environmental factors can be limited by the difficulty in reaching inaccessible areas, by the speed in which environmental variables change, and the cost and time incurred in collecting environmental data. Satellite data can be processed to produce surrogate measurements of meteorological parameters (Hay et al. 1996). For example, vegetation indices, cold cloud duration (CCD) techniques, soil and vegetation moisture, have been used to detect changes in vegetation, rainfall, and land surface temperatures respectively. For these reasons, remote sensing and GIS techniques are useful in studies of environmental and other epidemiological factors in vector-borne disease.

Belize is a country with an area of 23,000 square kilometers. Although small in land area, Belize differs by region in ethnicity, climate, elevation, disease, and vectors. Malaria is endemic in Belize (PAHO, Roberts et al. 2002b) and varies geographically by region. Historically, the western district, Cayo, and the southern district, Toledo, produced 50 percent or more of the malaria cases in Belize. In keeping with this pattern, in 1995, 63% of all malaria cases occurred in Cayo and Toledo. In 2000, the southern district of Toledo alone accounted for 45 percent of Belize's malaria cases. Up until, and through 1991, malaria control was achieved in Belize primarily through residual house spraying with the insecticide dichlorodiphenyltrichloroethane or DDT. During 1993 to 1995, countrywide spraying ceased in most areas due to budgetary constraints and pressures to abstain from the use of DDT even for public health purposes (Bangs 1999). Focal spraying was renewed sometime in 1995 due to a nation-wide increase of 60 percent or more in malaria cases. Vector control records for Corozal District indicate sporadic house spraying in certain villages in 1994.

In 1941, Kumm and Ram reported the presence of *An. vestitipennis* Dyar and Knab, and *An. albimanus* Wiedemann (Kumm 1941). Three anopheline species, *An. albimanus*, *An. darlingi* Root, and *An. vestitipennis*, are thought to be potential malaria vectors in Belize. The most widely distributed mosquito in Belize, *An. albimanus* is associated with cyanobacterial mats (CBM) and submersed-periphyton habitats (Rejmankova et al. 1993). In the northern coastal plains, larval densities were greatest in the dry season when the CBM increased in quantities in habitats that did not dry out. In the north, Komp reported the presence of *An. darlingi* in 1940, which was confirmed by

surveys conducted by Kumm and Ram in the Toledo and Stann Creek Districts (Komp 1940). Primarily a riverine anopheline, *An. darlingi* has been found during both wet and dry seasons in shaded or partly shaded patches of floating debris and submersed plants along creek and river margins (Manguin et al. 1996). Found throughout the year, *An. vestitipennis* is most abundant in the wet season in swamp forest and tall dense macrophyte habitats or TDM (Rejmankova et al. 1998, Roberts et al. 1993).

Belize is geographically and climatologically diverse (Barry 1992). Elevation varies from 0 to 20 meters in the marshes and swamp forests of the coastal plain to 1100 meters at the highest peak in the Maya Mountains. Annual rainfall varies from 1200 millimeters in the North to 4000 millimeters in the South. The wet season, also known as the hurricane season, begins in May and lasts through November. The dry season is from January through April. Average monthly minima range from 16^0C in the dry season to 24^0C in the wet season. Average monthly maxima range from 28^0C in the dry season to 33^0C in the wet season.

Several studies have used satellite imagery to characterize the habitats of the anopheline vectors in Belize (Rejmankova et al. 1993, Rejmankova et al. 1998, Rejmankova et al. 1995). Both unsupervised and supervised classifications of remotely sensed images have been used in Belize to predict anopheline densities. At high probability sites, using unsupervised classification of SPOT images, analysts predicted, with 50 percent accuracy, the presence of *An. pseudopunctipennis* along the Hummingbird Highway and *An. albimanus* in northern Belize with 89 percent accuracy (Rejmankova et al. 1995, Roberts et al. 1996). While using the SPOT image classifications to predict and verify riverine sites for *An. pseudopunctipennis* habitats,

Roberts et. al. (1993) collected *An. darlingi* larvae for the first time in 50 years in Belize. Satellite data also can be used, in conjunction with malaria case data, to examine relationships between environmental conditions and malaria cases.

Along with knowledge gained from vector studies in Belize, any correlation found between environmental factors and human malaria would improve the predictive capabilities of a malaria control program and generate hypotheses for further entomological studies. Preliminary results indicate a regional and seasonal difference in malaria transmission in Belize (Hakre 2003). Rainfall, temperature, land cover and demographic factors differ for northern, central and southern Belize. An investigation of the environmental factors accounting for the regional and seasonal differences would aid in prioritization of area-specific malaria control efforts by the National Malaria Control Program.

This retrospective study was designed to determine if groundcover, topography, distance to rivers and streams, distance inland, and weather data are associated with malaria risk. The study tested three hypotheses: 1) Higher rainfall is associated with higher malaria incidence in villages; 2) Shorter distance of villages to rivers is associated with higher malaria incidence in villages; 3) Higher percent of forest cover around villages is associated with higher malaria incidence in villages.

METHODS

STUDY POPULATION

This retrospective study examining environmental risk factors of malaria in Belize was conducted in villages that had malaria, census, and geographic location data for the period 1993-1995.

Village Location

A digital data set purchased from the Land Information Center (LIC) in Belize provided the geographic location of village centers. The data set was in ArcInfo coverage format. Village data assessed in the study were linked to the village location using the JOIN command in ArcInfo.

Malaria cases

Malaria cases during 1993 through 1995 for villages in Belize were extracted from the electronic database maintained by the Belize Ministry of Health's (MOH) National Malaria Control Program (NMCP). The database, which was started in 1989, is used to generate epidemiological reports. The years 1993 through 1995 were chosen since house spraying was minimal in most of the country.

Malaria cases in each village are detected by active and passive surveillance. In passive surveillance, villagers seek malaria diagnosis, through blood film examination, and treatment from a volunteer health collaborator (VC) in the village. During the 'malaria season,' personnel from the Vector Control Program (VCP) actively seek malaria cases by taking blood films from householders of malaria-positive fever patients. This action is termed active surveillance. The district VCP personnel conducting malaria control activities in the village collect the blood films taken by a VC. The blood films are

examined for malaria parasites at the district MOH laboratory by a technician/microscopist. All malaria positive films are sent to the central MOH laboratory microscopists for confirmation. The district VCP sends a weekly summary of positive slides and corresponding patient information to the NMCP. Patient information includes the name, age, sex, village of residence of the patient; the date the slide was taken, received and examined; the code of the person taking the slide (district VCP personnel/hospital personnel/village volunteer collaborator); the code of the technician who performed the microscopic examination; and species of malaria parasite. These data are entered into the national malaria database from which the malaria case information for this study was obtained. Patient identifiers were not used in this study since the malaria case information for the study was aggregated at the village level.

Malaria incidence/outcome

Malaria incidence per 1000 population was calculated for the 1993 to 1995 study period. Incidence per 1000 population was calculated using Belize's 1991 national census (Central Statistics Office, CSO), which provided the population size of a village. The assumption in using census data was that the entire population in each of the study villages was at risk for malaria.

RISK FACTORS

Environmental risk factors assessed in this study were averaged monthly daily total precipitation, averaged monthly daily minimum and averaged monthly daily maximum temperatures, distance to rivers and/or streams, distance inland, elevation, slope, and land cover. These data were obtained from several sources and are detailed below.

Weather data

Daily total precipitation, daily minimum temperatures and daily maximum temperatures for the study period were obtained for all study villages from the National Weather Service's (National Oceanic and Atmospheric Administration, NOAA) National Center for Environmental Prediction (NCEP), and from ground weather stations from the Belize National Meteorological Service (NMS)[1]. The NCEP data (Kalnay 1996, Kistler 2001) are a product of ground station data and satellite data using a General Circulation Model (GCM). The NCEP data are calculated globally and in a Gaussian grid. The grid is 1.875^{o} (longitude) by 2^{o} (latitude) in resolution. As the total area of Belize is much smaller than this grid, further interpolation was performed to obtain weather data for each village and ground weather stations. The finer resolution NCEP data set was obtained by performing a minimum curvature spline surface fit interpolation (Akima 1978 (a), Akima 1978 (b)).

The NMS weather station data had several limitations for use in this study. A few of the ground weather stations were initiated after 1995. Additionally, weather stations had data gaps where no readings were recorded. The sparse geographic coverage of the study area due to an insufficient number of ground weather stations (Appendix 6a) and gaps in readings at these stations (Appendix 6b) resulted in unstable interpolations of meteorological values for the study villages. Thus, the NCEP data sets were used in this study. The NMS ground weather station data were used to establish a baseline reference for the NCEP interpolations. Plotting the NCEP data interpolated for the ground weather station locations against the NMS ground station data enabled us to obtain adjustment

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[1] Twice daily temperature data were averaged per day and then per month. Daily total precipitation data were averaged per month.

calculations for the NCEP data set. Adjustments were made to the NCEP minimum and maximum temperatures by subtracting and adding –0.95^{0}C and 5.05^{0}C, respectively. Adding 32.44 millimeters adjusted NCEP precipitation data.

Distance to rivers/streams and distance inland

Rivers data (LIC, Belize) in ArcInfo coverage format (Appendix 3) for Belize were used to measure distance (in meters) to rivers and streams from the center point of a village. The NEAR command in ArcInfo was used to calculate the distances. The border of Belize was edited in ArcGIS to produce a 'coastline' coverage. The distance from the center of a village to the coastline of Belize (i.e. distance inland) was calculated in meters using the NEAR command in ArcInfo.

Elevation and Slope

Elevation data were calculated from the Global Land One-Kilometer Base Elevation (GLOBE) digital elevation data set (Appendix 7) produced by NOAA's National Geophysical Data Center or NGDC (http://www.ngdc.noaa.gov/seg/topo/globe.shtml). The PCI program, SLP, calculated the slope of each pixel by using the elevation value of surrounding pixels. The VIMAGE program in PCI added the elevation and the slope values to each village in the settlement layer. The settlement layer was exported to ArcView as a shapefile using the FEXPORT command in PCI. The slope and elevation values were obtained from the settlement shapefile table in ArcView.

Land cover

Land cover was assessed in this study by classifying a March 1994 30-meter resolution mosaic of two Landsat images (Appendix 8) and verifying the land cover

classes in the field. The 1994 mosaic excludes the northernmost district of Corozal and 12 villages in the bordering district of Orange Walk.

A 60-class unsupervised classification, using an isodata algorithm, was performed on the Landsat image (Appendix 9). The resulting classes were compared with land cover on 1:250,000 Belize topographical maps. Using a 60-class cluster in the algorithm split the land cover types that were similar on the topographical maps. A 30-cluster classification was performed next (Appendix 10) and checked against the topographical maps and a classification performed and verified in the field by Kevin Pope of a Year 2000 SPOT image for northern Belize. The class boundaries matched the land cover boundaries on the maps and SPOT classification. Using PCI software, a report of the area of each class in meters was generated for each two-kilometer village buffer zone.

Using photo-interpretation of the original Landsat image, an initial land cover type for each class was selected. Seventeen of the 30 classes predominated in the study villages. These were checked and modified during field trips to the two-kilometer buffer zones around 27 villages. These 27 villages were either in the top or bottom 30 percentile of malaria incidence villages during 1993 to 1995. Whenever possible, villagers were interviewed to ascertain the period of time land cover classes, such as cultivation, were in existence. Wright's 1959 vegetation map for Belize was used in the field as an aid in naming and developing broad categories of the land cover classes. Field verification of these classes showed a few of the classes represented similar vegetation types. Land cover classes that were similar among 17 classes were collapsed to produce 10 broad land cover categories: Forest (broadleaf and shrub), pine forest, agricultural

land, domestic cultivation, savannah, mangrove, marsh, urban, sea, and water (Appendix 11).

ANALYSES

Univariate

Descriptive statistics (mean, minimum, maximum, and standard deviation) were calculated for all variables assessed in the study (Table 3). Daily maximum and minimum temperatures and daily total precipitation values were averaged across three years by month to calculate average monthly daily maximum and average monthly daily minimum temperatures and average monthly daily total precipitation. To examine annual trends, daily minimum and daily maximum temperatures and daily total rainfall were averaged across months for each year of the study period[2]. The average annual temperatures and average annual total precipitation were examined for large-scale variation by looking at directional trends using ArcGIS Geostatistical Analyst extension (Appendix 12).

Temporal trends in meteorological variables and malaria incidence were examined using SAS version 8 for Windows. Temporal patterns in weather and average monthly malaria incidence during the study period were assessed by producing smoothed plots of monthly data for malaria incidence, temperature, and precipitation data averaged for all study villages beginning in May 1992 and ending in November 1995, which is the end of the wet season (Graphs 1-3). The smoothed plot was fitted to data using a spline routine implemented in SAS/GRAPH. This routine is a method for smoothing noisy data;

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[2] The values for the wet season prior to the study period were included to observe the lag in the effects of temperature and precipitation on malaria incidence.

the observed data points do not necessarily fall on the line. The smoothed line is a cubic spline that minimizes a linear combination of the sum of squares of the residuals of fit (a measure of how close the observed data points are to the smoothed line) and the integral of the square of the second derivative (a measure of the smoothness of the line) (Reinsch 1967). For these plots, a slightly greater weight was placed on goodness-of-fit (60%) than on smoothness of the line (40%).

Bivariate

Association between malaria incidence and environmental variables were examined. Prior to inclusion in bivariate analyses, average monthly malaria incidence, average monthly temperatures, and average monthly total precipitation for all study villages, averaged for the three years of the study period, were plotted against the log of malaria incidence to graphically examine for the presence of a linear relationship with incidence. Associations between malaria incidence and each weather variable were assessed using the GENMOD procedure in SAS version 8 for Windows. Similarly, association between malaria incidence and topographical variables was examined using the GENMOD procedure. Rate ratios, 95 percent confidence intervals, and p-values were calculated for each environmental risk factor. For variables with a temporal component, repeated and autoregressive statements were used in the GENMOD procedure (Appendix 13).

Multivariate

Multivariate analyses were performed using Poisson regression models to identify those variables most important in predicting malaria rates. Poisson regression was used

since malaria cases, the outcome in this study, are counts of events that occurred during a time interval per observation (i) or village and therefore, have a Poisson probability distribution (Agresti 1996). The Poisson regression model is a loglinear model where Y, the number of malaria cases, has a Poisson distribution and uses a log link function. The mathematical form of this model is: $\log \mu = \alpha + \beta x$ where μ=malaria incidence (i.e. log [cases/population]) and x=vector of explanatory variables. The explanatory variables were precipitation, temperature, elevation, distance of villages to rivers and the coast, and land cover variables. The GENMOD procedure in SAS version 8 for Windows was used to fit the Poisson regression model. The counts of malaria cases were adjusted to the population of the village by using the population as an offset on a logarithmic scale in the Poisson regression model.

Using repeated measure and autoregressive statements, all variables associated with malaria incidence with p-value < 0.25 and not highly correlated with each other were entered into a Poisson regression model for all villages (Appendix 13). The repeated measure statement was used in the multivariate analyses to indicate that a village or observation was included more than once (twelve times for each village during the study period) in the regression model. The autoregressive statement was used in model-building to account for a variable's temporal correlation in a village (i.e. the value of a time-dependent variable for a village may be closer in value to the subsequent month's value). Associations among variables individually associated with average monthly malaria incidence ($p<0.25$) were assessed using the MIXED MODEL procedure in SAS version 8 for Windows. Variables individually associated with malaria incidence ($p<0.25$) were precipitation, minimum temperature, elevation, distance of villages to

rivers, distance of villages to the coast, percent savannah, percent urban development, and percent forest. Among variables that were associated with each other, only percent forest cover was entered into the final model. This variable was chosen over distance of village to rivers because it was more significantly associated (individually) with average monthly malaria incidence ($p<0.05$). We ran the final model for smaller environmental areas (i.e. by district) by using average monthly malaria incidence and average monthly daily total precipitation for each year of the study versus a three-year averaged monthly value (Appendix 13b). Including monthly data for each year provided a larger sample size to run the model with precipitation and percent forest for smaller geographic areas. To give a more meaningful interpretation of precipitation and percent forest cover, point and 95 percent confidence intervals were calculated for a 10-unit change instead of changes per unit (Hosmer et al. 1989). The formula to calculate rate ratios per 10 units was: $\psi(10) = e^{(10 * \beta x)}$. The formula to calculate 95 percent confidence intervals for rate ratios was: $e^{(10 * \beta x \pm 1.96 * 10 * SE)}$ where SE is the standard error of the slope coefficient, β.

RESULTS

Table 1 shows the distribution of the study villages by district and by population. Cayo District had the highest number of villages (39) in the study followed by Toledo District with 36 villages. Stann Creek District had the least number of villages (22) in the study and was the least populated district with 16,291 people. Belize District was the most populated and had the settlement with the highest population (44,067 people). Corozal and Toledo Districts had the least populated villages in the study (14 and 25 people respectively).

Table 2 shows the distribution in malaria incidence by year of study. During 1993 through 1995, the highest annual malaria incidence occurred in 1994 when 27 villages had rates of 200 cases per 1000 population or more. The highest number of villages with no malaria (19) was in 1995. Figures 1, 2, and 3 display maps of Belize indicating the distribution of malaria incidence during the study period. During the three years, Toledo District had the highest incidence followed by Cayo and Stann Creek Districts. Corozal, Orange Walk, and Belize, the northern districts, had the lowest incidence.

Table 3 depicts descriptive statistics for all variables in the study. The highest elevation of a village was 496 meters. The lowest average monthly daily total rainfall in a village was 61.2 millimeters and the highest was 321.9 millimeters. The lowest average monthly daily minimum temperature was 17.1^0C and the highest average monthly daily maximum temperature was 36.0^0C.

Data exploration in ArcGIS's Geostatistical Analyst extension revealed trends in annual averaged daily minimum and daily maximum temperatures and annual averaged

daily total precipitation data (Appendix 12a-c). Maximum temperatures were highest in the north and decreased in a southerly (blue lines on y-axes, Appendix 12a1-3) and westerly direction (green lines on x-axes, Appendix 12a1-3). Minimum temperatures decreased in westerly (green lines on x-axes, Appendix 12b1-3) and southerly (blue lines on y-axes, Appendix 12b1-3) directions. Average annual daily total precipitation varied by year. In 1993, total rainfall was highest in the North with a decreasing gradient towards the southern district of Toledo and the western district of Cayo (Appendix 12c1). The 1993 rainfall pattern seen is atypical for Belize where southern areas have more rainfall than northern areas. In 1994, it rained more in the south and west, a normal pattern for Belize (Appendix 12c2). In 1995, it rained less in southern and eastern Belize than in northern and western Belize, which is another abnormal pattern for Belize (Appendix 12c1).

Graphs 1 through 3 are smoothed plots of malaria incidence versus average monthly temperature and average monthly precipitation by date (year, month) averaged across study villages. In general, for the country of Belize during 1993 through 1995, peaks in malaria incidence followed, with a time lag, peaks in temperature and precipitation. Graph 1 displays average malaria incidence and average minimum temperature by month. The moving averages of malaria incidence and minimum temperature are similar with a lag of at least one month seen between averages; as seen for average maximum temperature by month in Graph 2. However, the time lag in 1994 between the moving averages of malaria incidence and maximum temperature was much longer than that seen in 1993 and in 1995. Graph 3 displays average malaria rates and its correlation with average daily total precipitation by month. Peaks in average malaria

incidence follow peak average total rainfall much more closely in time than the moving averages of temperature. A lag of one to two months is seen between incidence and rainfall for 1993 and 1994 but not 1995. As a result of weather trends seen in Graphs 1 to 3, for bivariate and multivariate analyses, the weather variables were projected forward by one month so as to correlate average malaria incidence with the previous month's weather and not the weather in the same time frame as the occurrence of malaria cases. The moving averages of maximum and minimum temperatures were similar and therefore associated (Appendix 14). With the onset of rainfall, minimum temperatures increased as opposed to maximum temperatures, which decreased (Appendix 14).

Distances of villages to rivers/streams and the coastline, elevation, (Appendix 15) and five land cover (domestic cultivation, savanna, marsh, urban, fresh water) variables required log transformation for a linear relationship with the log of malaria incidence. Minimum temperature, precipitation, percent forest (Appendix 15), pine forest, mangrove, and agriculture, rose linearly (i.e. a unit increase in the weather variable, X, corresponded with an equal rise in Y, malaria incidence) with log of incidence. The six variables were assessed without transformation in calculating rate ratios in bivariate analyses (Table 4). Slope data, as calculated in PCI, were either values of 0 or 1. As such, slope in villages was treated as a categorical variable in the analyses.

The individual effect of environmental factors, during the study period, on average monthly malaria incidence average for the country is shown in Table 4. Average monthly daily total precipitation, average monthly daily minimum temperature, elevation, and percent forest in a village were individually associated with malaria incidence ($p<0.05$). An increase in precipitation (RR=1.0007; 95% C.L. – 1.0005,1.0009;

$p<0.0001$) and minimum temperature (RR=1.042; 95% C.L. – 1.036, 1.048; $p<0.0001$), higher elevation (RR=2.12; 95% C.L. – 1.2, 3.8; p=0.01) and more forest cover (RR=1.03; 95% C.L. – 1.02,1.05; $p<0.0001$) in a village were associated with higher malaria risk in villages. Average monthly maximum temperature did not vary much (range=7.1^{0}C) among villages, and therefore, its effect on malaria incidence could not be studied since the regression model with this variable would not converge.

Table 5 shows the association among the risk factors eligible for entry into multivariate analyses ($p<0.25$). Correlations among the multivariate-eligible variables, precipitation, minimum temperature, elevation, distance of villages to rivers and the coast, percent forest, urban development and percent savanna, were tested. As seen before (Appendix 14), precipitation was associated only with minimum temperature. Significant correlations existed among the other environmental variables ($p<0.05$).

Multivariate analyses

Precipitation and percent forest were entered into the final model. Statistical associations existed among the variables elevation, distance of villages to rivers and the coast, percent savanna, and urban development (Table 5), and therefore, were omitted from the final model. The final model included 179 villages (Table 6). Increase in precipitation per 10 millimeters (RR=1.008) and more forest cover per 10 percent (RR=2.16) were significantly associated with higher malaria incidence in a village ($p<0.0001$). Higher precipitation (per 10 millimeters) was significantly associated with higher malaria risk in villages in Toledo (RR=1.016; 95% C.L. – 1.008, 1.024; p=0.0003) and Cayo (RR=1.001; 95% C.L. – 1.0002, 1.001; p=0.01). Lower precipitation (per 10 millimeters) was significantly associated with higher malaria risk in Corozal (RR=0.990;

95% C.L. – 0.984, 0.996; $p=0.003$) and Orange Walk (RR=0.988; 95% C.L. – 0.988, 0.999; $p=0.02$). Higher percent forest was significantly associated with malaria risk in villages in Belize (RR=1.57; 95% C.L. – 1.44, 1.71; $p<0.0001$), Cayo (RR=1.67; 95% C.L. – 1.4, 2.0; $p<0.0001$), and Stann Creek Districts (RR=1.35; 95% C.L. – 1.13, 1.61; $p=0.001$).

DISCUSSION

Our hypotheses that precipitation and percent forest were associated with higher malaria incidence in villages were confirmed by analyses conducted in the study. Surprisingly, proximity of villages to rivers was not associated with higher malaria incidence in our study villages (p=0.17). As expected, the effect of precipitation and percent forest differed significantly by administrative district, which was chosen to represent the microenvironments within Belize in this study.

Precipitation was chosen over minimum temperature for consideration in the final model since it is a more likely determinant of vector growth, longevity, and habitat availability. Humidity and temperature are important environmental variables that determine the longevity of a vector. In a sub-tropical country like Belize, the higher temperature is typically conducive to vector growth and sporogony as temperatures always range above 15ºC (average minimum temperature in this study was 17.1ºC), the minimum ambient temperature necessary for growth of *P. vivax* (MacDonald 1952). Precipitation determines ambient relative humidity since days of heavy rainfall increase relative humidity in the environment. Furthermore, prolonged precipitation creates habitats for certain vectors or washes away larval habitats by increasing water current in rivers. Thus, precipitation is indirectly an influential factor in vector survival and therefore vector capacity.

Increased precipitation and higher percent forest were associated with higher risk of malaria in villages. In Belize, both rainfall and amount of forest cover are likely determinants of larval habitat availability in villages. As seen for *An. vestitipennis* and *An. darlingi* in previous entomological studies in Belize (Manguin et al. 1996,

Rejmankova et al. 1998), greater forest cover would provide more shaded habitats and also more detritus for larvae to shelter in and obtain nutrients.

In the multivariate regression models for microenvironments, higher precipitation was associated with increased malaria risk in Cayo and Toledo Districts. This relationship was seen graphically the two districts when precipitation (lagged by one month) and malaria incidence, summarized for all villages, were compared by each month of the study period (Appendix 16). High populations of *An. vestitipennis*, an important vector in Belize displaying endophagic behavior, were found in Toledo District after heavy rains during the wet season (Grieco 2000). During the study, heavy rainfall created optimum breeding habitats (clean water collecting in low-lying, shaded areas of forests with heavy canopy) that were either muddied or dried out in the dry season. Experimental hut studies, conducted in Toledo District to examine the house entry and exit behavior of *An. Vestitipennis,* indicated the vector entered houses more readily when conditions in the environment were inhospitable such as periods of increased rain and wind speed (Grieco et al. 2000). In another study conducted in Belize in 1996 by Rejmankova et al, larvae of *An. vestitipennis* were collected in the wet season and not in the dry season (Rejmankova et al. 1998). During the wet season, more habitats in southern Belize were positive for larvae than in northern Belize (38% versus 17%). Additionally in this study, *An. vestitipennis* larvae were positively associated with flooded forests (i.e. more tree cover and detritus) and tall dense macrophyte habitats. During the wet season (i.e. prolonged periods of high rainfall), higher forest cover near villages flooded with rain provided suitable habitats for *An. vestitipennis* and therefore increased malaria risk in southern villages.

During 1993 through 1995, in the northern districts of Corozal and Orange Walk, lower precipitation was associated with higher malaria risk in villages. *Anopheles albimanus*, a ubiquitous vector species in Belize, was collected in high densities in the dry season in both these districts (Rejmankova et al. 1993, Rejmankova et al. 1995). *Anopheles albimanus* is highly associated with cyanobacterial mat habitats found in marshes in Belize. The cyanobacterial mats increase in the dry season in marshes flooded from the rainy season and produce higher larval densities when compared to the wet season (Rejmankova et al. 1993). Entomological surveys have found *An. vestitipennis* in both the districts associated with tall dense macrophytes (*Typha)* found in marshes (Rejmankova et al. 1998). Higher numbers of this vector species were found in the wet season than the dry as larval habitats dried out. Additionally, larvae were found in habitats with lower water depths (mean water depth of 10.0 cm) than higher water depths (mean of 15.9 cm). It may be possible that immediately following periods of prolonged rainfall, increased water levels among *Typha* and less disturbance of water surfaces from steady rains promoted *An. vestitipennis* growth unlike during the height of the dry season when the *Typha* habitats dry out or the height of the wet season when the disturbed water surface may flush out habitats. It is likely that a decrease in heavy rains at the end of the wet season increased *An. vestitipennis* populations and subsequently increased malaria risk in northern villages. In the dry season, when *An. vestitipennis* habitats dried out, the accumulated water and cyanobacterial mats in marshes supported *An. albimanus* habitats in Corozal and Orange Walk and further increased malaria risk in the two districts.

Higher percent forest was associated with higher malaria risk in villages particularly in Belize, Cayo and Stann Creek Districts. *Anopheles darlingi* has been found in entomological surveys conducted in the three districts. *Anopheles darlingi* larvae and adults have been found in central Belize, specifically Cayo and Belize Districts (Manguin et al. 1996, Roberts et al. 1996). In the survey of the Sibun and Belize river systems of central Belize by Manguin et al, *An. darlingi* larvae were associated with floating wood detritus, and shaded, submersed *Cabomba* species vegetation habitats along river margins in central Belize. Furthermore, overhanging trees, especially bamboo, of secondary forests and submerged roots of the trees were found to provide shade for habitats and trap floating detritus in the river. The floating detritus provided camouflage for the larvae from predators. Komp, of 1940, found *An. darlingi* immatures and blood-fed adults (in houses) in the dry season in Stann Creek District (Komp 1940). In rural areas of Stann Creek District, Kumm and Ram found *An. darlingi* in the wet season at elevations of less than 330 meters (Kumm 1941). The vector studies conducted in the Cayo, Stann Creek and Belize Districts indicate *An. darlingi* breeds in shaded riverine habitats associated with forest cover, and may play a significant role in malaria transmission in these districts.

In enzyme-linked immunoassays testing for human *Plasmodium* circumsporozoite protein in field-collected mosquitoes, *An. darlingi* and *An. vestitipennis* have shown higher infection rates than *An. albimanus* (Achee et al. 2000, Grieco 2000). Both vector species display endophagic and anthropophilic behavior (Grieco 2000, Roberts et al. 1993, Roberts et al. 2002a). The flooded forest and tall dense macrophyte habitats of *An. vestitipennis* and the riverine habitat of *An. darlingi* (Manguin et al. 1996) are of greater

significance for malaria transmission in villages than the marsh habitats of *An. albimanus*.

Of the three vector species that transmit malaria in Belize, *An. albimanus* is less readily infected by malaria parasites and has shown exophilic and zoophilic feeding behavior in entomological surveys in Belize (Achee et al. 2000, Bangs 1999, Grieco 2000, Roberts et al. 2002a). Although it has been commonly found in all six districts in Belize, its characteristics of low infectivity by malaria parasites, and non-human feeding preference make it a less important vector in Belize. Therefore, the habitat with which this vector is associated is of less consequence than the habitats of the other important vectors of malaria in Belize.

Higher elevation was individually associated with higher malaria risk in villages. Elevation increases from 0 to 1100 meters heading from the coastal plains of Belize inland towards the Maya mountains located in southwestern Belize. Western and southern Belize have more forest cover and river systems than do the coastal plains. In this study, elevation in villages was correlated with distance to rivers, distance inland, and forest cover. In Belize, higher elevation in villages may be a proxy for higher percent forest and proximity to rivers, factors that provide suitable habitats for *An. darlingi* and *An. vestitipennis,* both important malaria vectors in Belize.

In general, for villages in Belize, proximity of a village to rivers or streams was not significantly associated with higher malaria risk. *Anopheles darlingi* has been associated with riverine habitats and either larvae or adult stages of this species have been found in all six districts in Belize. However, as a previous survey in Belize indicated, this species was easily found only in specific microenvironments where overlaps existed

between proximity to rivers (less than one km), elevation of less than 50 meters, and no forest cover between houses and larval habitats (Roberts et al. 1996). Results of another vector survey effort indicated *An. darlingi* presence/abundance were highest in Stann Creek villages than in Cayo or Toledo villages (Hakre 2003). It may be that only environmental conditions of certain villages located in specific geographic regions in Belize have increased malaria risk stemming from proximity to rivers or streams and explain the lack of association among study villages between high malaria risk, during 1993 through 1995, and short distances to rivers. The rivers data set used in this study visually indicates the Belize, Cayo, Stann Creek and Toledo Districts to have more river systems than the northern districts of Corozal or Orange Walk. Therefore, the northern districts possibly have less available habitats for *An. darlingi* than the other districts.

Malaria data used in the study were incident, and not prevalent, cases and were available by day of diagnosis and for every village. The use of incident cases provides a better idea of malaria occurrence over time, as it reflects the occurrence of new cases and not the total number of existing cases, which is the case with prevalence data. More accurate estimation of malaria incidence would have been calculated if population totals had been available for the years studied. This study covered three years and villages nation-wide and thus was able to adjust for temporal correlation in statistical analyses. Although we were unable to adjust for confounding factors such as population migration, vector abundance and species, and malaria control efforts such as sporadic focal spraying during 1994 and 1995, we were able to assess environmental risk factors for malaria in villages in different regions of Belize in a cost and time efficient manner. This was possible due to the power of developing and using a GIS by integrating land cover data

from a Landsat image, satellite weather data, elevation data from a DEM, and digital data sets of rivers and settlements in ArcInfo coverage formats.

House spraying with DDT for malaria control, in effect since 1950, had slowed in 1990 and 1991 and stopped in Belize in 1993 except for some areas in the northern districts where sporadic spraying continued from 1993 to 1995 (Bangs 1999). We chose to examine the effect of environmental risk factors on malaria incidence in villages during a period in Belize when minimal or no malaria control efforts (i.e., insecticide use) were exercised, which subsequently resulted in a historically significant surge in malaria cases. As a consequence, the association between environmental factors on malaria incidence rates in villages was more obvious because we avoided the confounding action of residual house spraying on malaria transmission rates and man-vector contact inside houses.

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Table 1
Study population

		Population		
District	No. of villages	Minimum	Maximum	Total
Corozal	33	14	7062	28561
Orange Walk	23	101	11014	29458
Belize	26	66	44067	53763
Cayo	39	54	8952	41804
Stann Creek	22	61	6435	16291
Toledo	36	25	1132	10113
Country	179	14	44067	179990

Table 2
Distribution of malaria incidence in 179 villages by district and year of study

	Malaria Incidence (per 1000 population)														
	0	<50	50 – 100	100 – 200	200+	0	<50	50 – 100	100 – 200	200+	0	<50	50 – 100	100 – 200	200+
District	1993					1994					1995				
Corozal	2	20	9	2	-	3	12	14	3	1	3	25	4	1	-
Orange Walk	0	18	2	3	-	1	16	4	1	1	2	19	1	-	1
Belize	6	13	6	1	-	6	14	1	1	4	7	14	2	2	1
Cayo	2	16	12	5	4	4	16	10	3	6	4	12	7	12	4
Stann Creek	1	15	2	2	2	2	12	-	3	5	1	9	4	1	7
Toledo	3	10	5	11	7	1	10	9	6	10	2	12	4	8	10
Country	14	92	36	24	13	17	80	38	17	27	19	91	22	24	23

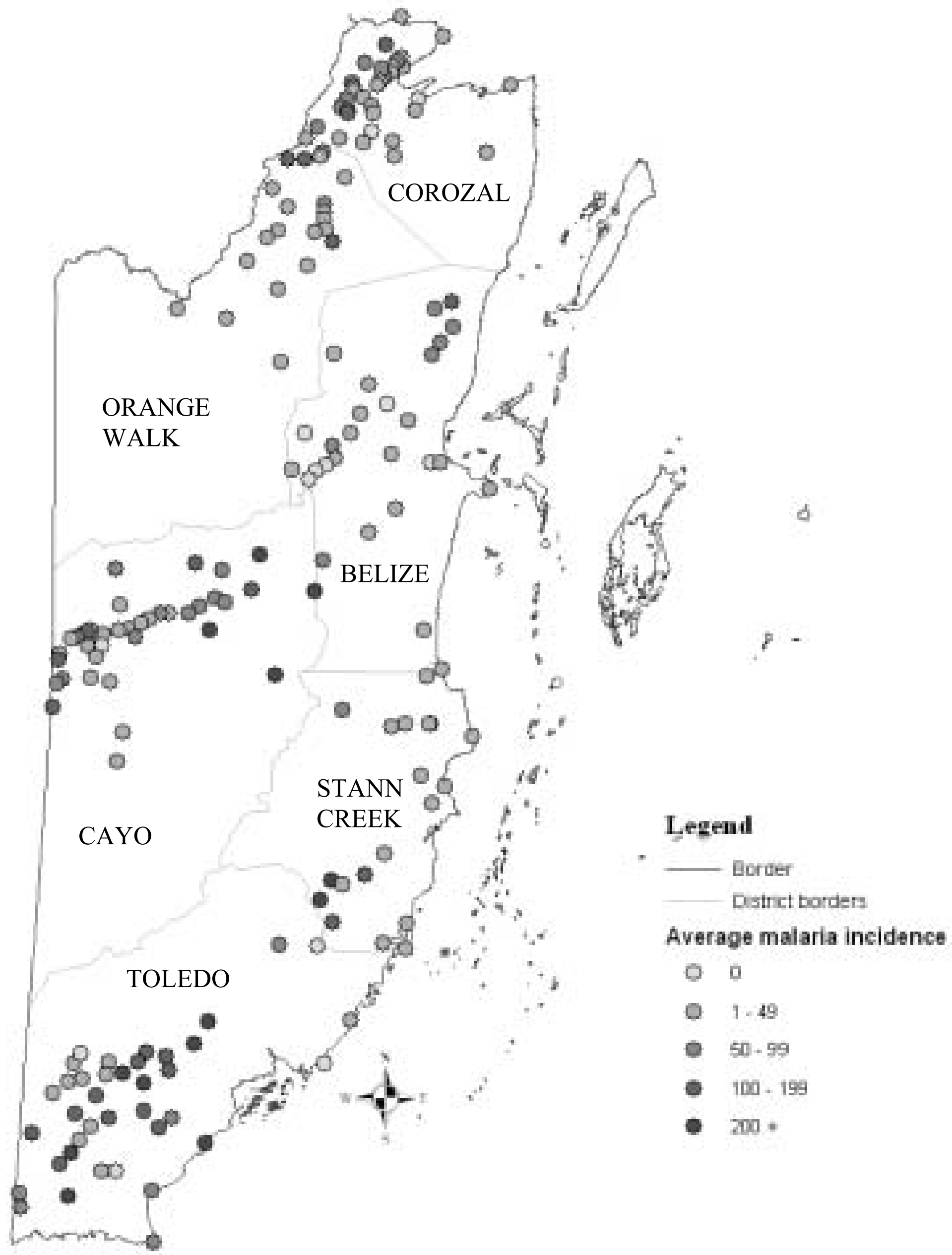


Figure 1: Malaria distribution (average annual incidence per 1000 population) in Belize in 1993

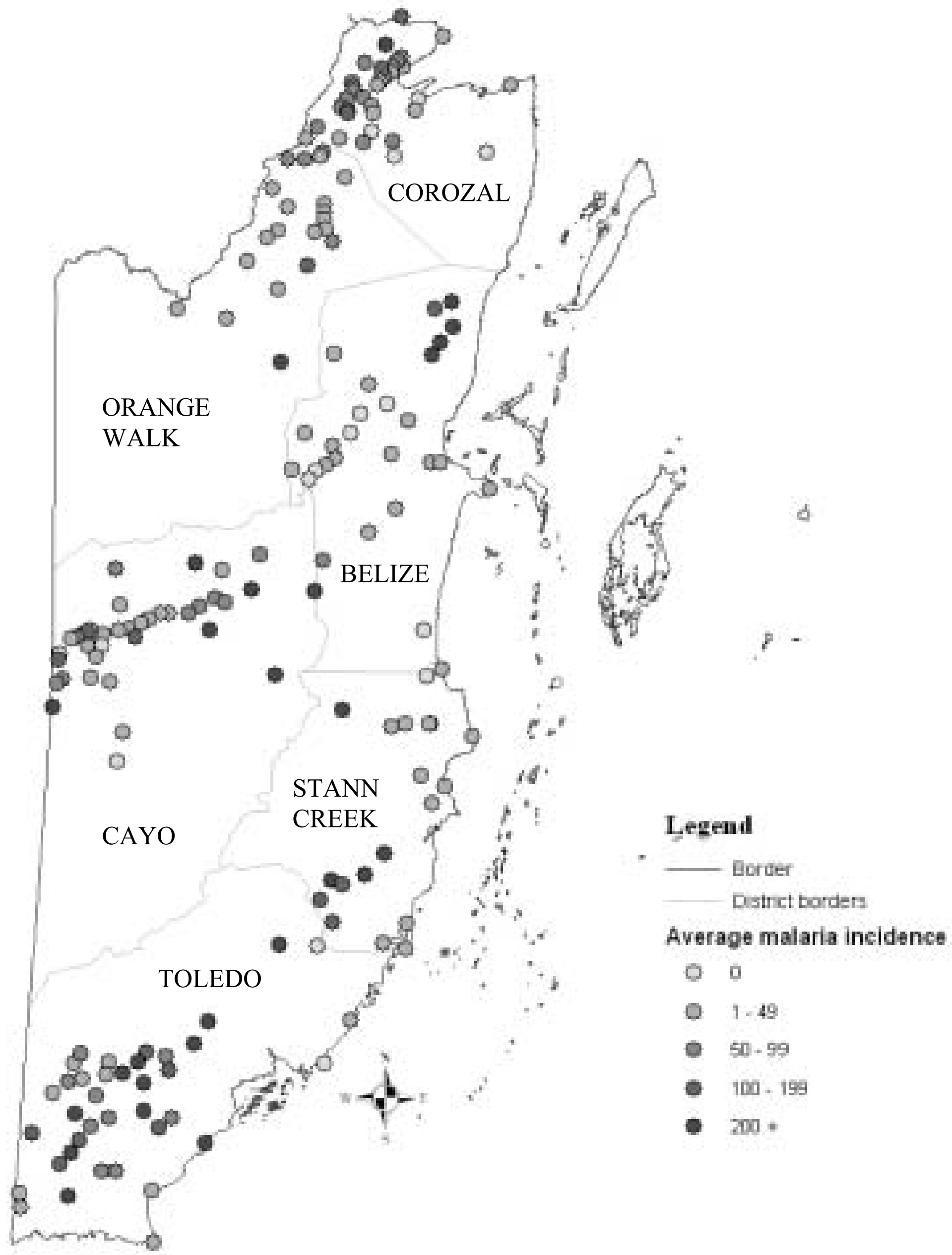


Figure 2: Malaria distribution (average annual incidence per 1000 population) in Belize in 1994

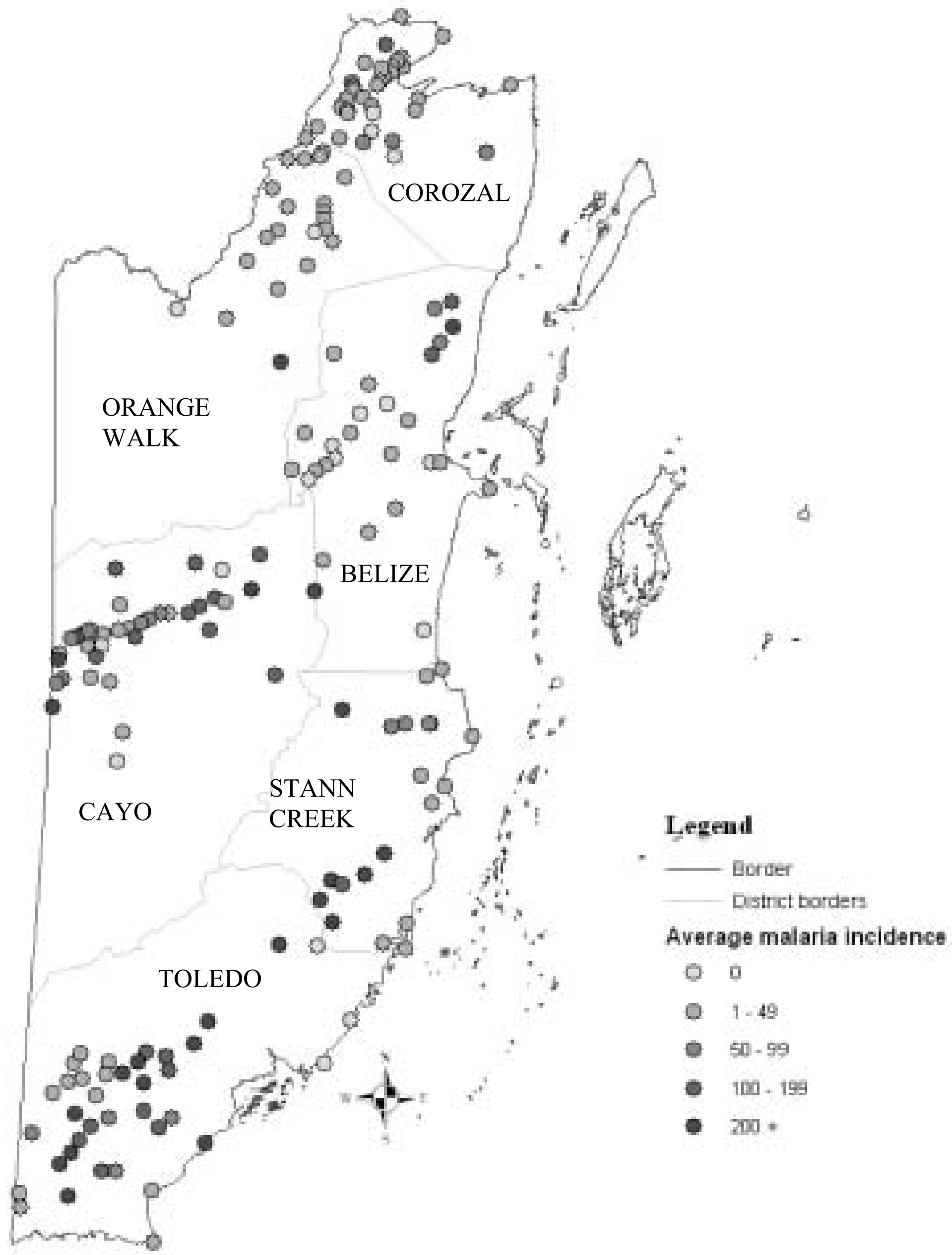
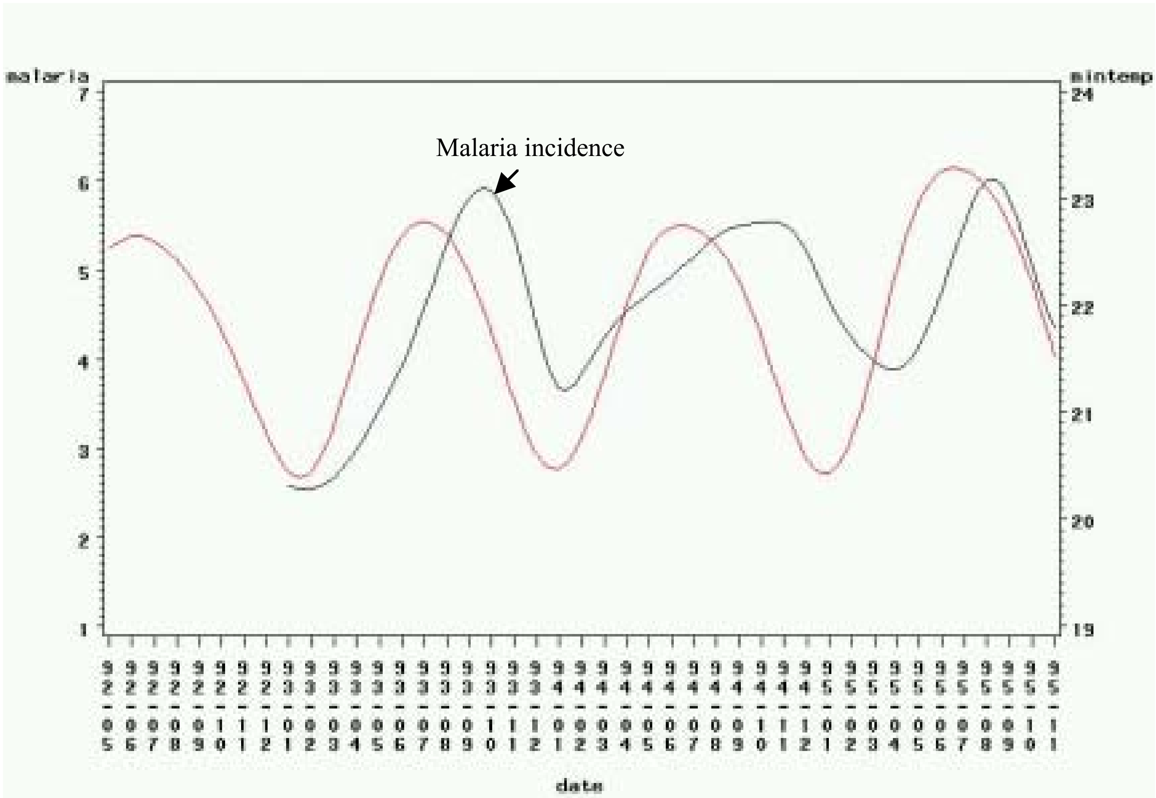


Figure 3: Malaria distribution (average annual incidence per 1000 population) in Belize in 1995

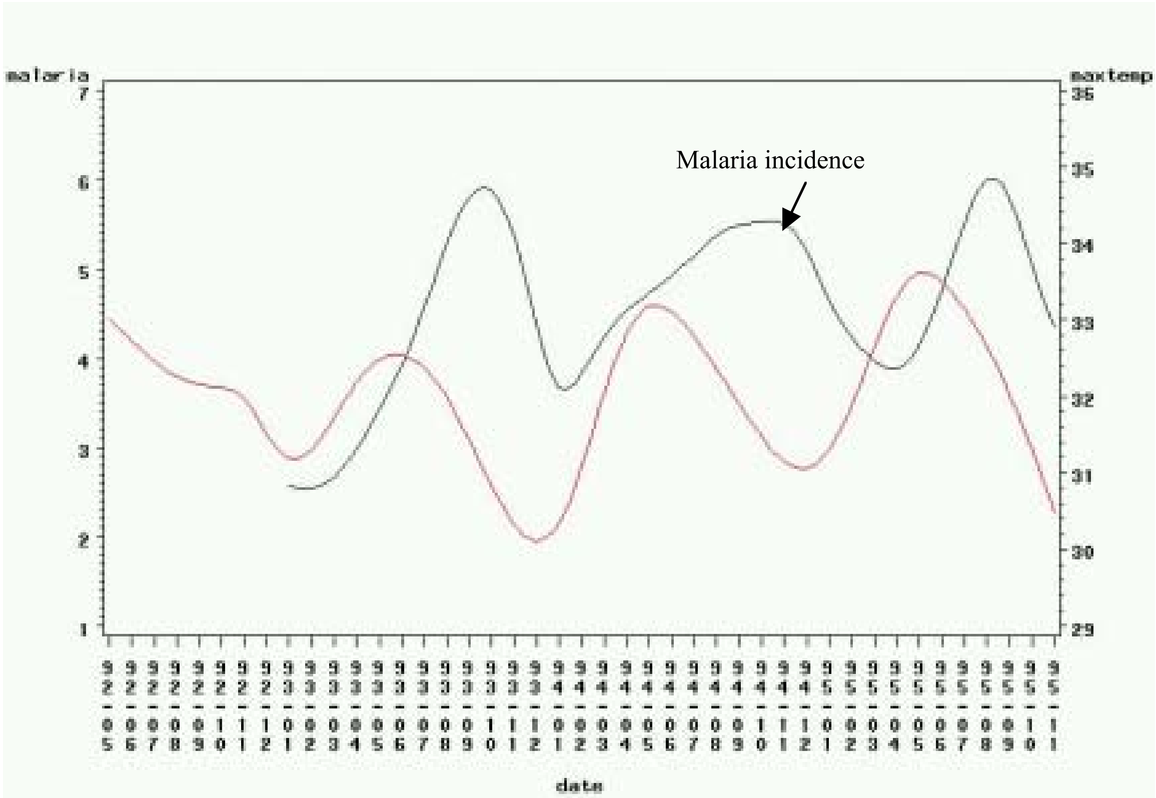
Table 3
Descriptive statistics of variables assessed in 179 villages

Variable	N*	Mean	S.D.	Minimum	Maximum
Malaria cases[@]	790	7	6	1	36
Population	179	1006	3551	14	44067
Topography					
Distance of village to rivers/streams (m)	179	1359	1731	1	7393
Elevation (m)	179	56	73	0	496
Distance of village to the coast (m)	179	29244	25861	89	91004
Slope	179	0.4	0.8	0	1
Weather[@]					
Precipitation (mm)	2148	180	69	61	322
Minimum temperature (^{0}C)	2148	22	2	17	25
Maximum temperature (^{0}C)	2148	32	2	29	36
Land cover (%)					
Agriculture	134	22	17	0.1	86
Domestic cultivation	134	1	5	0	38
Forest	134	33	28	0	93
Mangrove	134	2	2	0	14
Marsh	134	4	6	0.03	34
Pine forest	134	5	5	0.1	29
Savanna	134	3	6	0	38
Urban	134	10	7	0.4	39
Water	134	2	5	0	50

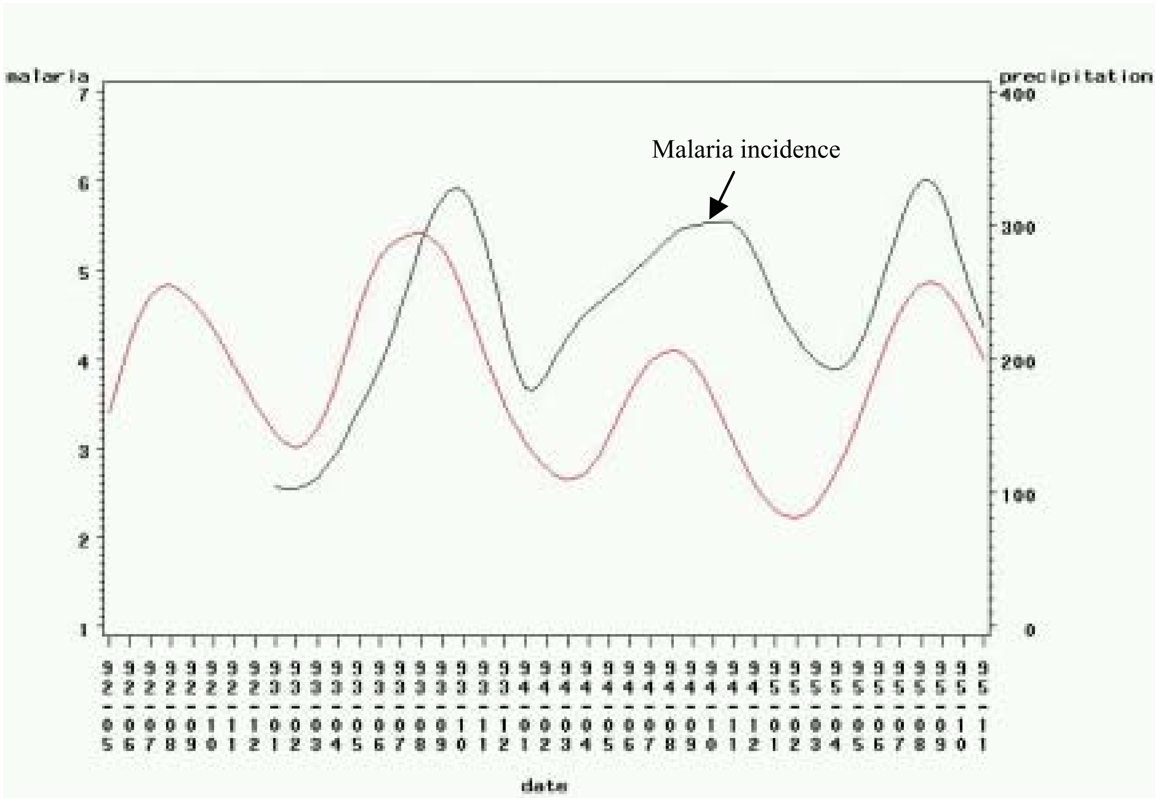
*N=Number of observations
@Data averaged by month across 3 years (1993-1995); for weather: n=179 villages x 12 months; for malaria cases: n=179 villages x no. of months each village had malaria cases



Graph 1: A smoothed plot of average monthly malaria incidence and minimum temperature by month during 1993 to 1995 in 179 villages, Belize



Graph 2: A smoothed plot of average monthly malaria incidence and maximum temperature by month during 1993 to 1995 in 179 villages, Belize



Graph 3: A smoothed plot of average monthly malaria incidence and precipitation by month during 1993 to 1995 in 179 villages, Belize

Table 4
The effect of environmental variables on malaria incidence in Belize during 1993 to 1995

Variable	RR	95% Confidence interval		p-value
Weather[@]				
Precipitation^ (mm)	1.0007	1.0005	1.0009	<.0001
Minimum temperature ^(^{0}C)	1.0417	1.0358	1.0478	<.0001
Topography				
Elevation*(m)	2.118	1.197	3.749	0.01
Slope of 1	1.468	0.530	4.068	0.46
Slope of 0	1.000	Referent		
Distance to rivers* (m)	0.629	0.325	1.219	0.17
Distance to coastline* (m)	1.748	0.942	3.245	0.08
Land cover (%)				
Agriculture	1.010	0.981	1.041	0.49
Forest	1.032	1.018	1.045	<.0001
Pine forest	1.040	0.893	1.212	0.61
Mangrove	0.753	0.460	1.234	0.26
Domestic cultivation*	1.079	0.399	2.918	0.88
Marsh*	0.534	0.098	2.925	0.47
Savannah*	0.449	0.133	1.518	0.20
Urban	1.046	0.971	1.127	0.24
Water*	0.937	0.261	3.360	0.92

[@] Variables are average monthly daily values averaged across 1993-1995
* Variables were log transformed prior to bivariate analyses
^ Analyses were performed on weather data pushed one month forward

Table 5

Risk factors individually associated with average monthly malaria incidence during 1993-1995

Variable	Precipitation @** (mm)	Minimum temperature @** (^{0}C)	Elevation * (m)	Distance to rivers/streams * (m)	Distance inland * (m)	Forest (%)	Savannah * (%)
Precipitation @** (mm)	-	-	-	-	-	-	-
Minimum temperature @** (^{0}C)	p<0.0001	-	-	-	-	-	-
Elevation * (m)	-	p<0.0001	-	-	-	-	-
Distance to rivers/streams * (m)	-	p<0.0001	p<0.0001	-	-	-	-
Distance inland * (m)	-	p<0.0001	p<0.0001	p<0.0001	-	-	-
Forest (%)	-	p<0.0001	p<0.0001	p<0.0001	p<0.0001	-	-
Savannah * (%)	-	p<0.0001	p<0.0001	p<0.0001	p=0.008	p<0.0001	-
Urban * (%)	-	p=0.73	p<0.0001	p=0.001	p<0.0001	p<0.0001	p=0.01

@ Variables are average monthly daily values averaged across 1993-1995
* Variables were log transformed prior to bivariate analyses
** Analyses were performed on weather data pushed one month forward

Table 6
Final Poisson regression model for the country (179 villages) and by district

Variable	Beta	Standard Error	Rate Ratio*	95% Confidence Limits*		P-value
Country						
Precipitation (mm)	0.0008	0.0001	1.008	1.006	1.010	<.0001
Forest (%)	0.0769	0.002	2.158	2.075	2.244	<.0001
By district						
Corozal						
Precipitation (mm)	-0.001	0.0003	0.990	0.984	0.996	0.003
Forest (%)	NA	NA	NA	NA	NA	NA
Orange Walk						
Precipitation (mm)	-0.001	0.0003	0.994	0.988	0.999	0.02
Forest (%)	-0.003	0.016	0.969	0.704	1.333	0.90
Belize						
Precipitation (mm)	-0.001	0.0004	0.994	0.986	1.002	0.12
Forest (%)	0.045	0.0044	1.568	1.439	1.710	<.0001
Cayo						
Precipitation (mm)	0.0007	0.0003	1.007	1.001	1.013	0.01
Forest (%)	0.0515	0.009	1.674	1.403	1.997	<.0001
Stann Creek						
Precipitation (mm)	-0.001	0.0005	0.993	0.983	1.003	0.19
Forest (%)	0.030	0.009	1.351	1.133	1.612	0.001
Toledo						
Precipitation (mm)	0.002	0.0004	1.016	1.008	1.024	0.0003
Forest (%)	-0.002	0.012	0.977	0.771	1.239	0.90

*Estimates are for 10-unit increments (for precipitation: per 10 millimeters; forest: per 10 percent).

CHAPTER 5

Manuscript 4

Household risk factors for malaria in two villages in Belize, Central America

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ABSTRACT

Malaria transmission differs among households in a village and among individuals within a household. The study objectives were to determine risk factors at the household level and to determine if malaria cases clustered within households in two villages in Belize. The studies in both villages used a case-control study design to examine the odds of malaria in an individual household.

The study population of the case-control studies was households surveyed by the Vector Control Program (VCP), Ministry of Health in San Martin, Cayo District, in 1997 and Red Bank, Stann Creek District, in 1998. Cases in both villages were defined as houses with one or more malaria cases during 1997, and controls were defined as houses with no malaria cases during 1997. Malaria history of households was ascertained by linking villager information to malaria cases, for 1993 through 1998, extracted from the National Malaria Database, Ministry of Health, Belize. The attempted human biting rate (HBR) of three primary vectors was calculated from mosquito landing collections conducted by the VCP in the two villages.

Demographic, domestic, and peri-domestic factors collected in the VCP survey were examined. Univariate, bivariate and multivariate analyses were conducted to determine risk factors for malaria case(s) in a house. Clustering of malaria cases within a household during 1993 through 1998 was investigated in both villages. In San Martin, four of 23 variables analyzed were significantly associated with malaria by case and control status. In Red Bank, five of 22 analyzed variables were significantly associated with malaria by case and control status. The most stable and predictive model retained three of 11 variables considered in the logistic regression models for San Martin and three of nine variables for Red Bank. In San Martin, during 1993 through 1998, three to eight percent of households had 50 % or more of malaria cases. Similarly, in Red Bank, 50% or more of malaria cases occurred in five to 12 percent of households. *Anopheles albimanus* attempted to bite most frequently in San Martin. In Red Bank, *An. darlingi* was most common and had the highest HBR.

Examining risk factors for the odds of malaria at the household level in both villages in Belize revealed that history of having malaria in any of the preceding four years was associated with occurrence of malaria during the study period. Other factors associated with the occurrence of malaria in a household were the proximity of a household to a stream, number of male or female occupants in a household, and the construction of outer walls. These results indicate that households with and without malaria can be similar and yet differ significantly within a village and between villages.

KEYWORDS: Malaria, household, clustering, stream, occupants

INTRODUCTION

Malaria transmission differs among countries, regions within a country, human settlements within regions, households within a village, and among individuals within a household (Greenwood 1989). Reasons for these variations range from proximity of humans to vector breeding sites (Ghebreyesus et al. 1998, Ghebreyesus et al. 1999, Gunawardena et al. 1998, Lindsay et al. 1995), vector characteristics such as abundance, endophagic behavior, and competency (Grieco 2000, Roberts et al. 1993, Roberts et al. 2002a, Snow 1987), housing design that promotes increased human-vector contact (Gamage-Mendis et al. 1991, Koram et al. 1995, Lindsay et al. 1990, Lindsay et al. 1988, Mendis et al. 1990, Schofield et al. 1984), presence of domestic animals near or within houses (Subramanian et al. 1991), behavior of household occupants (Lindsay et al. 1988), and genetic factors of an individual that confer immunity (Flint et al. 1986, Miller et al. 1977).

Belize is a malaria endemic country (PAHO, Roberts 2001). Before, during, and after the Global Malaria Eradication Campaign (beginning formally in 1957), Belize employed organized nationwide house spraying using the insecticide dichloro-diphenyl-trichloroethane (DDT), except for periods of cessation in 1965 and the 1980's due to limited funding, until the early 1990's (Brown et al. 1976, Roberts et al. 2002b). House spraying ceased during 1993 to 1996 except for focal spraying in areas of *Plasmodium falciparum* outbreaks and along the Mexican border. Malaria cases increased dramatically during this period of spraying cessation (Hakre 2003). A stratified approach to spraying that targeted villages at highest risk for malaria was initiated in 1996 and continued in 2000 (D.R. Roberts per. comm.). The Vector Control Program (VCP) in

Belize continued to use DDT for malaria control until 2000. Analyses by Roberts et. al. in 1999 of house spray data in Belize conducted during a period when all rural houses in Belize were sprayed showed that a minimum number of 28,380 houses needed annual spraying to prevent a rise in malaria cases. However, with the high costs of maintaining a spray program with an expensive substitute such as deltamethrin as well as increasingly limited human resources within the malaria control program, alternate malaria control approaches merit investigation.

Three *Anopheles* species, *An. albimanus, An. darlingi,* and *An. vestitipennis* are considered vectors of malaria in Belize. *Anopheles albimanus* is found ubiquitously in Belize, and is exophilic and zoophilic (Bangs 1999, Grieco 2000, Rejmankova et al. 1995). It has been found near rivers and among cyanobacterial mats in marshes (Manguin et al. 1996, Rejmankova et al. 1996). *Anopheles darlingi* has been found in shaded habitats along river margins that have floating detritus and submerged vegetation (Manguin et al. 1996). Collected commonly in indoor collections in Stann Creek and Toledo Districts, it has been found infected with malaria sporozoites (Kumm 1941). *Anopheles vestitipennis* larvae has been associated with tall dense macrophyte and flooded forest habitats (Grieco 2000, Rejmankova et al. 1998). In an experimental hut study in Toledo District, adults were determined to be highly endophagic and entered houses primarily through eaves, doors, and windows, and secondarily, through gaps in walls (Grieco 2000).

Analyses of malaria data over eight years in four villages in Belize, by Roberts et al in 1999, determined that a mere tenth of the households in a village produced more than 50 percent of the malaria cases in a village. Consequently, a malaria control

approach at the community or local level that focuses on high malaria risk houses within a settlement would maximize limited human and financial malaria control resources and potentially reduce the burden of malaria. Currently, financial and human resource constraints in control programs limit prevention and control efforts to villages or communities at highest risk. Consequently, high risk individuals or families residing within a community or village at low risk are overlooked by disease prevention and control efforts. Therefore, identifying household risk factors for malaria would help malaria control programs in selectively targeting the high-risk households in all villages for prevention and control (surveillance, chemotherapy and house spray) thereby reducing disease and costs to the disease control program.

We examined risk factors at the household level in two villages in Belize, namely San Martin in Cayo District and Red Bank in Stann Creek District (Figure 1). Both villages had the highest malaria incidence in their corresponding administrative region or district (National Malaria Database, Ministry of Health, Belize). In both villages, using a case-control study design, we examined demographic, and household factors associated with malaria risk in families. Additionally, to assess malaria risk within households in the two villages, we examined the malaria case history of each household during 1993 through 1998.

METHODS

The subjects of the case-control studies were the households in San Martin in Cayo District, and Red Bank in Stann Creek District surveyed by the Vector Control Program (VCP), Ministry of Health in 1997 and 1998. San Martin is located in the eastern foothills of the Maya Mountains and on the outskirts of Belmopan, the capital of Belize. Its residents work primarily in Belmopan as laborers or domestic workers. Most residents immigrated from other areas in Belize or from neighboring Central American countries (i.e., Guatemala, Honduras, or El Salvador).

Red Bank lies in the western foothills of the Maya Mountains and 15 miles from the eastern Caribbean coast. Broadleaf forests border the village in the west, and pine forest and savannah surround it in the east. Its predominantly Mopan Maya residents originally migrated from the southern district of Toledo in 1982 (Vector Control Program, Ministry of Health). The men primarily farm neighboring foothills and occasionally work on nearby banana plantations while the women take care of the household and children (Everett et al. 1993).

Cases in both villages were defined as houses with one or more malaria cases during 1997 and controls were houses with no cases of malaria during 1997. History of malaria in households during the study period was ascertained from the Belize Ministry of Health's (MOH) National Malaria Database. Information, regarding families and households, assessed in this study were obtained from the village mapping and census surveys conducted by the VCP in San Martin and in Red Bank during 1997 and 1998, respectively.

Village census

This study used four village census computerized databases obtained from the VCP. Both San Martin and Red Bank villages had databases with household occupant and house information. The databases were a result of surveys by the VCP team during August 30, 1997 through November 30, 1997 in San Martin, Cayo District and during April 21 through April 29, 1998 in Red Bank, Stann Creek District. Prior to conducting the surveys study investigators trained and evaluated team members, in August 1996, on how to gather information on the questionnaire forms (Appendix 18). Additionally, surveyors from the Lands Department (Ministry of Natural Resources, Belize) trained the survey team in land survey techniques using a GARMIN hand-held global positioning system (GPS) unit and a base station. The survey team interviewed occupants, numbered, mapped, and through direct observation, recorded the construction of each house in the village and within a one-kilometer radius from the outskirts of the village. The coordinates of one house in the village were obtained using a GPS unit and were corrected using a base station. Other houses in the village were mapped using standard surveying techniques. The information of each surveyed house in the village was entered into two separate databases. The first database contained information regarding the house number, house coordinates, house construction, environment surrounding the house within 20 meters (i.e. peri-domestic environment), and domestic animals owned by the occupants. The second database comprised house occupant information (last and first names, age, sex, occupation and job location).

The house coordinates obtained from the VCP database were displayed on SPOT images taken on March 14, 1997 (covered Red Bank) and April 15, 1999 (covered San

Martin). The house coordinates and village area on the images did not match (Appendix 19) possibly because of inaccuracies in the first GPS readings in the villages or using a different datum. To rectify the discrepancy, in December 2001, new coordinates were taken of a proportion of the houses with occupants still living in the same location as during the 1997 and 1998 surveys. Of the 175 houses in San Martin with coordinates from the 1997 census, 74 (42%) houses were re-mapped. In Red Bank, of 81 houses with census coordinates, 16 (20%) were re-mapped. Reasons for not re-mapping houses in San Martin and in Red Bank were non-existence of the census house or the presence of new tenants, no occupants, or no one present at home during the re-mapping process. Re-mapped house coordinates demonstrated that the offset was systematic, and the new coordinates were used to adjust the original coordinates.

The latitude and longitude of all coordinates in the original census for both villages were adjusted. In San Martin, the original census coordinates were adjusted by subtracting one and 197 for the Easting (latitude) and Northing (longitude) coordinates respectively. In Red Bank, adding 6.50 to the Easting coordinates and subtracting 104.38 from the Northing coordinates adjusted the original census coordinates. The house coordinates were re-displayed on the SPOT image. The adjusted coordinates visually matched the village location on the image (Appendix 20).

For each village, the house and house occupant databases were merged using the house number common to both databases. After joining the databases, the mean age, the number of total occupants, and the number of female and male occupants per household were calculated.

Malaria History

All malaria cases that occurred in both villages during 1993 to 1998 were extracted from the National Malaria Database (NMD). The NMD contains a record of all blood films positive for malaria for the entire country of Belize. Volunteer collaborators in each village and VCP personnel from the village's administrative district take blood films through active and passive surveillance. The collected blood films are submitted to the microscopists at the district MOH laboratory. All blood films positive for malaria are reported on a weekly basis to the central VCP's National Malaria Control Program office. These weekly reports are entered into a national computerized database and contain the last and first name, age, sex, malaria species, and dates the film was collected and diagnosed for malaria, village code, surveillance method, and microscopist's code. During 1993 through 1997, the NMD had a record of 548 cases for Red Bank, and 1293 cases for San Martin. The malaria cases were matched to villagers by last and first names, age and sex. Each villager in both databases was assigned a unique study identification number. The study identification number was assigned to the corresponding malaria case identified as a match. After linking malaria cases to villagers, the names were stripped from the linked databases to maintain confidentiality. The database containing house occupants (villagers) information and linked malaria information was merged with the database containing house coordinates and other information.

Distance to streams

Coordinates were taken along the banks of streams in Red Bank and in San Martin at locations where the stream was accessible by foot. The field coordinates and SPOT images of both locations were used to digitize the streams in ArcView 8.1.0. The coordinates taken along streams in both villages were displayed on the images to increase accuracy while digitizing. The digitized streams were then converted to coverage files in ArcInfo and, using the NEAR command, distance of houses to streams in Red Bank and San Martin were calculated in meters per house.

Attempted Human Biting Rate

The human biting rate (HBR) was calculated to determine the density of each of the three main vector species attempting to bite exposed adults indoors and outdoors during each season in the two villages. The vector information was obtained from surveys the MOH teams conducted during August 21, 1996 through November 14, 1997. January through June were considered the dry season and July through December the wet season. During the wet season of 1996 and the dry and wet seasons of 1997, the teams conducted indoor and outdoor landing collections from 1600 to 1800 hours at houses in 32 villages in Cayo, Stann Creek, and Toledo Districts. San Martin and and Red Bank were included in the survey, and eleven and ten evening landing collections were made in each village, respectively. The adult HBR per person per two hours of landing collection for the wet and dry seasons by district were calculated by the formula below:

$$HBR_{district} = \frac{\text{Total number of adult female mosquitoes collected during each landing collection}}{\text{Total number of collectors at each landing collection}}$$

Analyses

Case and control (houses with no malaria cases in 1997) houses were displayed in ArcView 8.1.2 to examine for spatial clustering of malaria houses (Figures 2 and 3). Additionally, malaria case clustering within households was assessed for the period 1993 through 1998. The households producing 50 percent or more of malaria cases during 1993 through 1998 were displayed. Malaria incidence rate was calculated for each village using NMD case information and the VCP survey, which provided population information by age and gender.

Risk factors assessed in this study (detailed in Tables 2 and 5) were peri-domestic environment, house construction, house occupants' demographic information, domestic animals and distance to stream nearest to the villages. The mean values of continuous variables were compared for case and control (non-case) houses, with statistical significance determined using Student's t-test for independent samples. The continuous variables were malaria history of houses, house occupant's age, total number of occupants, number of male and female occupants, distance from the house to the stream, number of doors, number of windows, number of doors and windows screened, and the percent cover of environment around the house. For all continuous variables, the assumption of linearity in the logit (log odds ratio) was checked to ensure that the risk factors were used in the scale appropriate for the logistic regression model. Categorizing continuous variables into equal groups and plotting the mean of each group against the proportion of cases (houses with malaria) for each group checked the linearity assumption. Risk factors that did not have a linear relationship with the outcome were categorized into two to three levels (Tables 3 and 7). The logistic regression model

follows the form $g(x) = \log (\pi (x)/ 1 - \pi (x) = \beta_0 + \beta_1 x)$ where x is the risk factor or independent variable and g(x) is the logit transformation or the conditional mean of the outcome, Y, given the risk factor, x (Hosmer-Lemeshow, 1989). Additional bivariate analyses such as odds ratios, 95% confidence limits, and p-values were calculated for each variable, comparing each level to a referent ('normal') level.

Multivariate analyses were performed using stepwise multivariate logistic regression models to identify those risk factors associated with case houses or most important in independently predicting houses with malaria. Stepwise logistic regression sequentially considers each variable and arrives at a final parsimonious model consisting of the variables that are best associated with being a case or that best predict being a case, as opposed to a control. In the logistic model, variables were represented as indicator ('dummy') variables, where each category was coded 0 or 1 to represent the level's absence or presence, respectively. Only variables associated with being a case house at a p-value of less than or equal to 0.20 were entered into the logistic regression models for consideration in the final model. Data management, univariate, bivariate and multivariate analyses were conducted using the SAS System for Windows Version 8 and/or SPSS 11.0 for Windows. ArcView version 8.1.2 was used to conduct the spatial analyses in this study.

RESULTS

The case and control status of households in both villages were established by linking the malaria cases in the NMD to house occupants (Table 1, 2). For 1993 through 1997, 28 percent (356 malaria cases) were linked to villagers in San Martin (Table 1) and 65 percent (354 malaria cases) to villagers in Red Bank (Table 2). For 1997 alone, 31 of 78 cases (40%) in San Martin were linked to villagers and for Red Bank, 38 cases of 68 malaria cases (56 %).

Tables 3 and 4 show the selection of the houses in San Martin and Red Bank respectively. Of the 204 houses surveyed in San Martin by the VCP, 23 were case houses and 181 were control houses (Table 3). Four (17%) of case and 26 (14%) of control houses were excluded, since house coordinates or house occupant information were missing. Nineteen (11%) of 23 eligible case houses were included and 155 (89%) of 181 control houses were included in the study to total 174 case and control houses as the study population. In Red Bank, 98 (24 cases, 74 controls) houses were surveyed by the VCP in 1998. Of these 78 (20 cases, 58 controls) houses were eligible for inclusion in the study. Four (17%) case houses and 16 (22%) control houses were excluded, since house coordinates or house occupant information were missing from the VCP databases.

Tables 5 and 6 depict descriptive statistics (number, minimum, maximum, mean, standard error, p-value of comparison of means Student's t-test) of the risk factors assessed, by case and control status, in San Martin and Red Bank. In San Martin, malaria case houses and non-malaria control houses were identical in the mean number of female occupants (3.0 versus 3.0). Both case and control houses also shared similar house surroundings: percent of bare ground around the house (21.1 versus 14.6), percent of

trees around the house (18.2 versus 18.7), percent of shrubs around the house (15 versus 15.8), and percent of grass around the house (44.2 versus 47.5). House construction similarities such as number of doors (2.1 versus 2.0), number of windows (4.2 versus 3.8), number of doors screened (0 versus 0.1), and number of windows screened (0.7 versus 0.6) also were shared by both groups of houses.

In San Martin, more of the case houses had occupants of a lower mean age (18.8 versus 22.2), a higher average total number of occupants (7.1 versus 5.5), and a higher number of male occupants (4.2 versus 2.8). Additionally, malaria case houses had a higher number of years being a case house during 1993 through 1996 (1.7 versus 0.9), were located significantly closer to the stream in the village (223.3 meters versus 390.9 meters), and had less water around the house (1.4 % versus 3.7%).

In Red Bank, malaria case houses and non-malaria control houses shared similar distances to the stream in the village (156.4 meters versus 173 meters) and similar surroundings around the house (% grass: 44.1 versus 41.8; % shrubs: 15.8 versus 17.9; % trees: 20.2 versus 20.3; % bare ground: 19 versus 18.9; and % water: 1.0 versus 0.4). The case and control houses shared a similar mean age per household (16.8 versus 21.6 years) and number of female occupants per household (3.5 versus 2.8).

Tables 7 (San Martin) and 8 (Red Bank) show the frequency distribution, odds ratios, and 95% confidence limits for risk factors examined in relation to malaria case and non-malaria control houses. In San Martin, four of the 23 variables analyzed were significantly associated with malaria by case and control status. Distance of house to a stream (OR=1.01), history of having a malaria case(s) in the house within the past four years (2.0), total number of occupants per house (OR=1.3), and number of male

occupants (OR=1.6) were associated significantly with the odds of malaria occurrence in a household. The risk factors distance of a house to a stream, number of occupants and number of male and female occupants in addition to mean age and history of malaria in a household were left continuous in scale in the analyses as the values increased linearly with an increase in the proportion of malaria case houses (i.e. the two risk factors met the linearity assumption for use in logistic regression models). Domestic animals (poultry, cats, dogs), surroundings around the house (percent of grass, bare ground, shrubs, trees, water), kitchen location, type of stove and house construction (number of windows and doors, screened windows and doors, type of inner and outer roofs or walls, and floors) were not associated with malaria by case and control status ($p>0.05$).

Table 8 shows five of 22 analyzed variables in Red Bank were significantly associated with malaria by case and control status. Three variables considered continuous in scale in the analyses, household size (OR=1.2), number of male occupants per household (OR=1.4), and history of being a case house in the past four years (OR=1.8) were significantly associated with being a malaria case house in 1997 ($p<0.05$). Number of female occupants per household (OR's: 2-6.3) were associated significantly with the odds of malaria in a house. Type of outer walls was significantly associated with malaria in a house with houses made of sticks/ sticks and planks having 3.85 greater odds of being a case than a house constructed only with concrete or plank walls. Distance to stream from a house, surroundings around the house (grass, shrubs, trees, water, bare ground), domestic animals (horses, cats, dogs, chickens), outer roof, and floor type were not associated with malaria by case or control status ($p>0.05$).

Multivariate analyses for each village were conducted using stepwise logistic regression models. Different estimation methods, different sets of variables, and different methodology (forward versus backward) all consistently led to the final models presented in this study. In San Martin, the most stable and predictive model retained three of 11[*] variables considered in the logistic regression models (Table 9). The risk factors retained as associated with malaria by case and control status were history of being a case house in the past four years, male occupants in the household, and distance of a house to a stream. The model correctly predicted 75% of the malaria houses (sensitivity) and 67% of the non-malaria houses (specificity) using a cut-off value of 0.1. The Hosmer and Lemeshow Goodness of Fit test's Chi-square was 6.7, with 8 df and a p-value of 0.60 which indicated that the null hypothesis of good agreement between the observed and expected values of cases was accepted (Hosmer et al. 1989).

In Red Bank, the most stable model kept three variables that were associated with malaria case houses from the full model with 8[*] variables (Table 10). Though eligible (p=0.20), the variable 'chickens' was omitted from entry into the full model because of model instability due to zero values when entered along with the variable 'cats.' The risk factors of significance in the final model were history of being a case house in the past four years, number of female occupants in the household, and outer walls of the house. Using a cutoff value of 0.20, the model correctly predicted 84% of malaria houses (sensitivity) and 68% of non-malaria houses (specificity) among houses in the study in

[*] Total number of occupants, male occupants, case history, mean age per household, distance to streams, other animals, poultry, % grass around the house, number of doors, floors, inner walls

[*] Total number of occupants, female occupants, male occupants, mean age per household, history of being a case house in past 4 years, outer walls, cats, and number of doors

Red Bank. The Hosmer and Lemeshow Goodness of Fit test's Chi-square was 7.1, with 7 df and a p-value of 0.40, which signified the model fit the data well.

Table 11 and Figures 4 and 5 display the proportion of households within San Martin and Red Bank that had most malaria cases during 1993 through 1998. The results shown in Table 11 are from all the households surveyed by the VCP team. Figures 4 and 5 include only those households with geographic locations (i.e., the study population for the case-control studies). In San Martin, a mere three to eight percent of households accounted for 50 percent of malaria cases in the village. In Red Bank, 50 percent or more malaria cases occurred in a mere five to 12 percent of all households.

The results of indoor and outdoor landing collections conducted in San Martin and Red Bank are shown in Table 12, while the mean human biting rate, by wet and dry seasons, of three potential vectors in the two villages is shown in Table 13. Higher numbers of *An. albimanus* were collected in San Martin (35) than in Red Bank (8). *Anopheles darlingi* was more common in Red Bank (53) than San Martin (1). *Anopheles albimanus* attempted to bite most frequently in both seasons in San Martin (HBR_{DRY}: 0.2; HBR_{WET}: 2.1). *Anopheles darlingi* (HBR_{WET}: 0.10) and *An. vestitipennis* (HBR_{DRY}: 0.10) were collected as well in San Martin. In Red Bank, *An. darlingi* attempted to bite most frequently in both seasons (HBR_{DRY}: 1.3; HBR_{WET}: 2.5) followed by *An. albimanus* (HBR_{DRY}: 0.1; HBR_{WET}: 0.5).

DISCUSSION

In examining risk factors at a household level in two villages, we have shown that the malaria risk in a household significantly varies by age, gender, house characteristics, and vectors between villages. In both villages examined in this study, previous history of malaria and gender of occupants were associated with and predictive of malaria in a household. Because Belize's malaria control program has routinely collected and recorded malaria case information at the individual level since 1989, the control program has documentation at the household level of whether malaria transmission occurred during the year or in preceding year(s). Targeting only houses with malaria cases for malaria control efforts, such as house spraying, case treatment, and further reduction of human-vector contact through health education would translate into both reducing the malaria burden among the high risk populations in the community and saving scarce funds for the malaria control program.

Risk factors analyzed in the study were at the household level in both villages and were tied to concurrent malaria incidence data obtained from the Ministry of Health. The existence of a computerized national database of malaria positive blood films and corresponding demographic information enabled us to examine the history of malaria in a household. Use of a case-control design allowed us to assess a range of risk factors for a single outcome, malaria. Additionally, being able to establish the case status of a household (compiled from a national database) independent of the collection of risk factor information from a village census eliminated potential differential selection bias of study participants.

We were unable to link all malaria cases in the National Malaria Database (NMD) to villagers during the study period. VCP records indicate that Red Bank had 135 houses with a population of 565 in 1997 (Appendix 17). Our study included 78 houses resulting in a 58% inclusion rate. The census was conducted in Red Bank and not outlying areas. The cases recorded in the NMD as originating from Red Bank and the number of houses counted by the VCP may be from areas farther than one kilometer from the outskirts of the village. We were unable to determine if the houses not included in the census were significantly different from the houses included in our study since we did not have any information on houses not within the census.

In San Martin and Red Bank, factors not associated with increased malaria risk in a house were presence of animals, kitchen location, type of stove, numbers of windows or doors (except Red Bank), screened versus unscreened doors or windows, construction of inner roof or outer wall (except Red Bank), type of floor, peri-domestic environmental factors.

The risk of malaria increased significantly with more male and female occupants (in Red Bank only) in the household. In San Martin, after adjusting for other risk factors, number of male occupants was associated with malaria houses. The incidence rate in 1997 for male house occupants (38 cases per 1000 population) was more than twice that in females (16 cases per 1000 population) (Table 14). Male villagers aged 10 to 14 years (95 cases per 1000 males) had the highest mean incidence followed by males aged 35 to 39 years (69 cases per 1000 males). Accordingly, malaria risk was greater in a house with more male occupants. Most of the villagers in San Martin worked within the same district or administrative region and did not significantly differ in location of occupation

by malaria case or control status. However, males were primarily laborers, while females were domestic workers. The higher incidence among males may be due to their routine lifestyle and/or occupation. Males aged 35 to 39 may have had jobs that placed them more in the vicinity of other malaria-infected individuals (and therefore, infected vectors) and/or placed them in greater contact with vectors outdoors such as *An. albimanus.* In an experimental hut study conducted in Caledonia, Corozal District, *An. albimanus* exhibited peak biting activity two to three hours after sunset. Vector surveys in San Martin indicated *An. albimanus* was most common. Males may have been bitten more during peak biting hours than females due to greater exposure to vectors stemming from different gender-assigned roles within a household. In the early evening hours, females in the household may have been more active with domestic chores and provide mosquitos with fewer opportunities for landing and blood feeding.

In Red Bank, a higher number of male occupants and three to four female occupants were significantly associated with malaria case houses in bivariate analyses. However, in the final multivariate model predicting malaria in households, five or more female occupants conferred greater risk of malaria in a household (OR=3.9; p=0.06) though it was below the level of statistical significance. Across all ages, malaria incidence rate was slightly higher among females than among males in Red Bank (Table 14: 63 cases per 1000 males versus 66 cases per 1000 females). A likely explanation may be that men spent less time at home because of farming activities or working on nearby banana plantations while domestic chores kept women with young children occupied in and around homes more than men. Additionally, since indoor plumbing was absent in the village and water was supplied from community wells (Everett et al. 1993),

many Mayan women in Red Bank performed domestic chores such as washing in rivers and streams. Females were placed in greater contact with vectors and vector habitats around the domestic environment and therefore were at greater risk of malaria than males. In Red Bank, males and females aged 35 to 39 appeared to have had much higher risk of malaria than other adult age groups (1 case in 8 people; 250 cases per 1000 population). The small population size of this age group (8) makes the rate unstable. However, it is also possible that men and women in the 35 to 39 year old age group (as opposed to younger women/mothers) had greater contact with other communities through working in banana or citrus industries. It is possible that this population contracted the infection elsewhere and imported malaria into the community.

In both San Martin and Red Bank, in unadjusted or bivariate analyses, the odds of malaria increased significantly with more occupants. This may reflect the increased risk of malaria due to the confounding effect of more male and/or female occupants. However, it may independently be a risk factor as well. Studies conducted in Ethiopia, Cameroon, and the Gambia, suggest overcrowding is a risk factor for malaria (Ghebreyesus 2000, Koram et al. 1995, Kuate Defo 1995). In a study examining risk factors for malaria among children in Kilifi District, Kenya, malaria risk was higher in a child who had shared a room with another child with malaria (Snow et al. 1998). An infected vector within a household may infect multiple individuals, which may be facilitated by many people within few rooms. Although we were not able to distinguish whether the number of occupants in a house was a reflection of the number of rooms in the house (i.e., house size) or simply a reflection of overcrowding since information on the size of a house was lacking from the village census, while field-checking house

locations in Red Bank and from prior field studies, houses were mostly single-room homes that served as sleeping, kitchen, and living quarters.

In both villages, the mean age of case households was lower than control households in univariate analyses. However, in Red Bank, the statistical significance of the difference in mean age between case and control households was marginal ($p=0.06$). In both villages, 54 percent of villagers were 14 years of age or younger. Malaria incidence rates, by age, differed in the two villages. In San Martin, males aged 10 to 14 years had the highest malaria incidence (95 cases per 1000 males) of all age groups in 1997. In Red Bank, household occupants four years or younger had the highest malaria incidence in 1997 (males: 140 per 1000 males; females: 138 per 1000 females). In the both the villages, the higher malaria incidence among younger age groups may explain the initially significant results of younger age in case households.

In Red Bank, children aged four years or younger had the highest malaria incidence in 1997 (males: 140 per 1000 males; females: 138 per 1000 females). Furthermore, landing collections in the village in 1996 and 1997 indicated *An. darlingi* was the most common and abundant vector. These results suggest that *An. darlingi* transmitted malaria within households in Red Bank. These findings support previous study results that showed children aged four or younger had higher *P. falciparum* infections, and that *An. darlingi* was common in Stann Creek District (Hakre 2003). Preliminary observations from experimental hut studies along the Sibun River indicate *An. darlingi's* peak biting times are approximately 8:30 to 9:00 p.m., an hour before midnight, and around 5:00 a.m. (N. Achee, per. comm.). Children aged four or less may have greater exposure to *An. darlingi* than other age groups. In other Mayan villages,

young children have been observed in cloth pouches or cradles that are slung over a mother's head or back or inside the house (Grieco, per. comm.). As the children are immobile in the cradles, they are unresisting human bait for female *Anopheles* seeking a blood meal. Furthermore, young children have been observed to be generally less clothed than older children and as a result may be more exposed to mosquitos.

In both villages, having had malaria in the household in any of the preceding four years was associated with and predictive of malaria occurrence in a household in the study period. Characteristics of the house and its occupants, such as occupation; age-associated malaria risk; house construction, which increased risk of malaria in the household in any given year continue over time to place the household at high risk. In San Martin, only three households categorized as a case house had no malaria in preceding years. Of the remaining 15 case households, five households had malaria once before, while eight or more households had malaria at least two years or more. In Red Bank, of 20 case households, only two had no malaria prior to 1997. Of the remaining 18 case households, five had malaria once previously, and five had malaria in two of the years preceding the study. Seven or more households had malaria for three or more years prior to 1997. Clearly, in both communities certain households have much greater risk of malaria over time than others. Thus, if malaria control personnel had specifically targeted only malaria case households (households that had reported malaria cases in a prior year) for spraying in 1997, then they would have significantly reduced the malaria burden, during 1993 through 1997, in Red Bank by 79 percent (284 cases of 362 cases) and in San Martin by 82 percent (292 cases of 357 cases).

In addition to temporal clustering, across years, of malaria in households, we found that malaria cases clustered within households in a given year. In examining the malaria case history of households in the two villages during 1993 to 1998, we found that most malaria cases (50 percent or more) occurred in only three to 12 percent of housholds. This finding confirms observations of this phenomenon in another Belizean village (Carter et al. 2000). During 1989 to 1996, in San Pedro village in the southern district of Toledo, eight percent of the houses accounted for more than 50 percent of malaria. The clustering of malaria cases within houses has also been observed in studies conducted in Sri Lanka and Peru (Gamage-Mendis et al. 1991, Guthmann et al. 2002). The occurrence of one case within a household probably initiates a ripple effect of infections within that household and surrounding households. The characteristics and abundance of the primary vector transmitting malaria in a village are probably important determinants of the magnitude and velocity of the spread of malaria infections. Vectors such as *An. darlingi* and *An. vestitipennis* are competent vectors due to their house-entering and anthropophilic characteristics, especially when compared to the exophilic and zoophilic characteristics of *An. albimanus* (Grieco et al. 2000). As seen in this study, Red Bank, which had a higher density of *An. darlingi* and housing construction that increased accessibility to mosquitoes, had more than twice the malaria incidence rate of San Martin (64 cases versus 27 cases per 1000 population, respectively) in 1997. Malaria control efforts such as house spraying of known high-risk households as well as immediate and complete treatment of individuals infected with malaria within high-risk households would significantly decrease the malaria burden in local communities and consequently, the country.

In San Martin, proximity of a house to a stream increased the risk of malaria in a household. The location of a household close to a stream is a risk factor because the stream serves as a likely site for female blood-fed *Anopheles* adults to lay their eggs, as well as a site that produces adult mosquitoes. Both *An. darlingi* and *An. albimanus* were collected in San Martin, and it is probable that the stream that traverses the village was the breeding site for the two vectors. In a previous study conducted in central Belize, higher numbers of *An. albimanus* and *An. darlingi* adults were collected at houses located within one kilometer of a river (Roberts et al. 2002a).

Studies conducted in the Suba District of Western Kenya found significant variation in the abundance of *An. gambiae* based on the distance of larval habitats to the closest house (Minakawa et al. 1999, Minakawa et al. 2002). Most of the *Anopheles* adults in that study were collected within houses that were less than 300 meters of the closest larval habitat. Studies conducted in Peru, Ethiopia, and the Gambia indicated proximity of a vector breeding site conferred greater risk of malaria infections (Clarke et al. 2002, Ghebreyesus et al. 1999, Ghebreyesus 2000, Guthmann et al. 2002).

In a Sri Lankan study, houses with incomplete walls and ceilings located near water had a much higher malaria risk than houses with better construction (Gunawardena et al. 1998). In Red Bank, all houses in the study were located within 550 meters of the stream in the village, which is within the two-kilometer flight range of a vector. As the houses with malaria were not significantly closer to the stream than the houses without malaria cases, distances to the stream was not studied in multivariate analyses. Thus, under those circumstances, proximity to river was not a prominent risk factor.

In Red Bank, construction material of the outer walls was predictive for the risk of malaria in a house. Houses with walls of only sticks or sticks and planks had an almost 8 times greater risk of malaria than houses made of concrete or concrete and plank walls. As these houses were within one kilometer of the stream, the gaps in house walls made of sticks and/or planks likely facilitated vector entry and therefore increased human-vector contact. As gaps in house walls enabled mosquitoes to easily enter, factors such as two or more doors in houses (OR=2.6; p-value > 0.05) and unscreened windows were not significant risk factors in Red Bank. In a study of six Sri Lankan villages, Gamage-Mendis et al. (1991), found the risk of malaria was almost three times higher for families living in houses with incomplete mud or cadjan palm walls compared to families living in better constructed houses(Gamage-Mendis et al. 1991).

In summary, examining factors for the risk of malaria at the household level in two villages in Belize revealed that a history of having malaria in any of the preceding four years was associated with the occurrence of malaria during the study period. Malaria cases were highly clustered within households during 1993 through 1997. Other factors associated with the occurrence of malaria in a household in one of two villages were the distance of a house from a stream, number of male or female occupants in a household, and construction of outer walls. The risk of malaria among households and between villages differed significantly. The effectiveness of malaria control efforts, in planning and executing control strategies, in the Cayo and Stann Creek Districts might be enhanced if differences among and within villages were investigated further and accommodated. Diagnosing, treating, and spraying high-risk households within villages

might significantly prevent malaria burden and reduce operational costs in the two communities and probably other villages as well if similar measures were carried out.

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Table 1
Breakdown by year of malaria cases extracted from the National Malaria Database (NMD)* for San Martin

Description	1993	1994	1995	1996	1997	Total
NMD cases	385	338	337	155	78	1293
Number of cases linked	101	88	92	44	31	356
% Linked	26	26	27	28	40	28

*A total of 1313 cases were extracted from the NMD; twenty cases were excluded because they were duplicates; the remainder (1293) were used in linking cases to San Martin household occupants

Table 2
Breakdown by year of malaria cases extracted from the National Malaria Database (NMD)* for Red Bank

Description	1993	1994	1995	1996	1997	Total
NMD cases	172	55	99	154	68	548
Number of cases linked	98	34	67	117	38	354
Proportion linked (%)	57	62	68	76	56	65

*A total of 556 cases were extracted from the NMD; eight were excluded because they were duplicate cases; the remaining cases (548) were used in linking cases to Red Bank household occupants

Table 3
Selection of study population from census conducted in San Martin during September 30, 1997 through November 30, 1997

Characteristic	Number of houses in San Martin, Cayo District					
	Malaria houses (cases)		Non-malaria houses (controls)		Total	
	n	(%)	n	(%)	n	(%)
Houses eligible	23	(11)	181	(89)	204	(100)
Houses excluded	4	(17)	26	(14)	30	(15)
Houses included	19	(83)	155	(86)	174	(85)
Occupants eligible	160	(14)	976	(86)	1136	(100)
Occupants excluded	25	(16)	120	(12)	145	(13)
Occupants in the study	135	(84)	856	(88)	991	(87)

*Houses were excluded due to lack of coordinates and 1 control house had no occupants

Table 4
Selection of study population from census conducted in Red Bank during April 21, 1998 through April 29, 1998

Characteristic	Number of houses in Red Bank, Stann Creek District: Malaria houses (cases) n	(%)	Non-malaria houses (controls) n	(%)	Total n	(%)
Houses eligible	24	(25)	74	(76)	98	(100)
Houses excluded*	4	(17)	16	(22)	20	(20)
Houses included	20	(83)	58	(78)	78	(80)
Occupants eligible	180	(30)	417	(70)	597	(100)
Occupants excluded	29	(16)	87	(21)	116	(19)
Occupants in the study	151	(84)	330	(79)	481	(81)

*Houses were excluded due to lack of coordinates and/or villager information.

Table 5
Descriptive statistics of potential risk factors in malaria case and non-malaria control houses, San Martin, 1997

	CASES				CONTROLS				
VARIABLE	N[†]	Mini-mum	Maxi-mum	Mean (Std. Error)	N†	Mini-mum	Maxi-mum	Mean (Std. Error)	p-value[*]
Mean age per household (years)	19	12	33	18.8 (1.1)	155	9	77	22.2 (1.0)	0.02
Female occupants	19	1	4	3.0 (0.2)	141	0	7	3.0 (0.1)	0.7
Male occupants	19	1	8	4.2 (0.4)	153	0	7	2.8 (0.1)	0.0004
Household occupants	19	3	12	7.1 (0.5)	155	1	13	5.5 (0.2)	0.01
No. of years a case house in past 4 years	19	0	4	1.7 (0.3)	155	1	4	0.9 (0.1)	0.001
Distance to stream (meters)	19	61.2	372.3	223.3 (27.9)	155	7.1	390.9	161.5 (7.9)	0.001
Bare ground around house (%)	19	5	85	21.1 (5.6)	151	0	100	14.6 (1.4)	0.1
Grass around house (%)	19	5	65	44.2 (3.9)	151	0	90	47.5 (1.6)	0.5
Shrubs around house (%)	19	0	25	15 (1.6)	151	0	75	15.8 (0.7)	0.6
Trees around house (%)	19	0	30	18.2 (2.3)	151	0	35	18.7 (0.7)	0.8
Water around house (%)	18	0	10	1.4 (0.7)	152	0	35	3.7 0.6	0.01
Screened doors	17	0	0	-	145	0	2	0.1 (0.03)	0.4
Screened windows	17	0	7	0.7 (0.5)	145	0	16	0.6 (0.2)	0.9
Doors	17	2	3	2.1 (0.1)	145	1	4	2.0 (0.04)	0.2
Windows	17	0	10	4.2 (0.7)	145	0	16	3.8 (0.2)	0.5

[†] N = #(%) non-zero
[*] *p-value of comparison of means t-test

Table 6
Descriptive statistics of potential risk factors in malaria case and non-malaria control houses, Red Bank, 1998

	CASES				CONTROLS				
VARIABLE	N†	Mini-mum	Maxi-mum	Mean (Std. Error)	N†	Mini-mum	Maxi-mum	Mean (Std. Error)	p-value*
Mean age per household (years)	20	5	27	16.8 (1.2)	57	3	78	21.6 (2.2)	0.06
Female occupants	20	1	7	3.5 (0.3)	58	0	11	2.8 (0.3)	0.19
Male occupants	20	1	8	4.1 (0.4)	58	1	7	2.9 (0.2)	0.01
Household occupants	20	3	12	7.6 (0.6)	58	1	14	5.7 (0.4)	0.02
No. of years a case house in past 4 years	20	0	4	2.0 (0.3)	58	0	4	0.3 (0.1)	0.01
Distance to stream (meters)	20	16.9	319.7	156.4 (19.9)	58	2.6	548.2	173.0 (16.1)	0.58
Bare ground around house (%)	20	5	65	19.0 (3.9)	57	0	60	18.9 (1.8)	0.98
Grass around house (%)	20	10	70	44.1 (4.2)	57	0	90	41.8 (3.1)	0.66
Shrubs around house (%)	20	0	25	15.8 (1.5)	57	0	60	17.9 (1.6)	0.31
Trees around house (%)	20	5	39	20.2 (2.0)	57	0	50	20.3 (1.3)	0.98
Water around house (%)	20	0	5	0.9 (0.4)	57	0	5	0.4 (0.2)	0.26

† N = #(%) non-zero
* *p-value of comparison of means t-test

Table 7

Frequency distributions, unadjusted odds ratios, and 95% confidence limits (95% CL) for risk factors examined in relation with malaria case and non-malaria control houses, San Martin, 1997

Variable	Cases n=19 #	(%)	Controls n=155 #	(%)	Odds Ratio	P-value	95% CL	
Mean age per household					1.0	0.2	0.9	1.0
Occupants					1.3	0.02	1.0	1.5
Female Occupants					1.0	0.8	0.7	1.3
Male Occupants					1.6	0.001	1.2	2.1
Number of years a case house in past 4 years					2.0	0.002	1.3	3.0
Distance to stream (meters)					1.01	0.02	1.001	1.01
Number of doors					1.5	0.5	0.5	4.5
*Other animals^*								
Present	16	84	106	68	2.8	0.2	0.6	12.7
Absent	2	11	37	24	1.0		Referent	
Missing*	1	5	12	8	-	-	-	-
Poultry								
Present	10	53	56	36	1.9	0.2	0.7	5.2
Absent	8	42	87	56	1.0		Referent	
Missing*	1	5	12	8	-	-	-	-
Cat								
Present	5	26	43	28	1.1	0.8	0.4	3.3
Absent	13	68	100	65	1.0		Referent	
Missing*	1	5	12	8	-	-	-	-
Dog								
Present	13	68	83	54	1.9	0.3	0.6	5.6
Absent	5	26	60	39	1.0		Referent	
Missing*	1	5	12	8	-	-	-	-

^ The variables poultry, cat, and dog are combined. * No observations were recorded in the census.
'-' represents no value.

Table 7 (cont.)

Frequency distributions, unadjusted odds ratios, and 95% confidence limits (95% CL) for risk factors examined in relation with malaria case and non-malaria control houses, San Martin, 1997

Variable	Cases n=19 #	Cases n=19 (%)	Controls n=155 #	Controls n=155 (%)	Odds Ratio	P-value	95% CL	
Kitchen location								
Inside	11	58	58	37	2.2	0.2	0.8	6.3
Outside	6	32	69	45	1.0	Referent		
Missing*	2	11	28	18	-	-	-	-
Type of Stove								
Fire	9	47	71	46	0.9	0.8	0.3	2.5
Gas	8	42	54	35	1.0	Referent		
Missing*	2	11	30	19	-	-	-	-
Water around house (%)								
≤5	17	90	122	79	2.1	0.3	0.5	9.5
>5	2	11	30	19	1.0	Referent		
Missing*	-	-	3	2	-	-	-	-
Grass around house (%)								
≤45	11	58	61	39	2.	0.2	0.8	5.3
>45	8	42	90	58	1.0	Referent		
Missing*	-	-	4	3	-	-	-	-
Shrubs around house (%)								
>20	4	21	23	15	1.5	0.5	0.5	4.9
≤20	15	79	128	83	1.0	Referent		
Missing*	-	-	4	3	-	-	-	-
Number of windows								
Absent	4	21	17	11	1.6	0.5	0.4	6.2
Present	13	68	128	83	1.0	Referent		
Missing*	2	11	10	7	-	-	-	-
Screened Windows								
None	15	79	134	87	0.6	0.6	0.1	3.0
1-16	2	11	11	7	1.0	Referent		
Missing*	2	11	10	7	-	-	-	-
Floor type								
Dirt	11	58	64	41	1.9	0.2	0.7	5.0
Concrete, wood	8	42	89	57	1.0	Referent		
Missing*	-	-	2	1	-	-	-	-

* No observations were recorded in the village census. '-' represents no value.

Table 7 (cont.)

Frequency distributions, unadjusted odds ratios, and 95% confidence limits (95% CL) for risk factors examined in relation with malaria case and non-malaria control houses, San Martin, 1997

	Cases n=19		Controls n=155					
Variable	#	(%)	#	(%)	Odds Ratio	P-value	95% CL	
Outer roof								
Thatch	3	16	21	14	1.3	0.7	0.4	4.0
Cardboard, rubber	5	26	34	22	1.3	0.8	0.3	4.9
Metal	11	58	96	62	1.0	Referent		
Missing*	-	-	4	3	-	-	-	-
Inner roof								
Absent	14	74	126	81	0.6	0.4	0.2	1.9
Present[@]	5	26	29	19	1.0	Referent		
Missing	-	-	-	-	-	-	-	-
Outer walls								
Planks, sticks	15	79	129	83	1.3	0.6	0.4	4.3
Concrete	4	21	26	17	1.0	Referent		
Missing^	-	-	-	-	-	-	-	-
Inner walls								
Celotex, cloth, none	9	47	16	10	0.3	0.1	0.1	1.1
Planks, sticks	6	32	36	23	0.8	0.8	0.2	3.1
Concrete	4	21	103	67	1.0	Referent		
Missing^	-	-	-	-	-	-	-	-

* No observations were recorded in the census

[@]Cardboard, celotex, metal, rubber rye, thatch, wood

'-' represents no value

Table 8

Frequency distributions, unadjusted odds ratios, and 95% confidence limits (95% CL) for risk factors examined in relation with malaria case and non-malaria control houses, Red Bank, 1998

Variable	Cases n=20 #	(%)	Controls n=58 #	(%)	Odds Ratio	P-value	95% CL	
Total occupants per house					1.2	0.03	1.0	1.4
Male occupants per house					1.4	0.02	1.1	1.9
History of being a case in past 4 years					1.8	0.02	1.1	2.9
Mean age per household					1.0	0.23	1.0	1.1
Female Occupants per house								
5+	3	(15)	11	(19)	2.1	0.37	0.4	11.0
3 – 4	13	(65)	16	(28)	6.3	0.005	1.8	22.5
0 – 2	4	(20)	31	(53)	1.0	Referent		
Type of Stove								
Fire	19	(95)	52	(90)	1.8	0.35	0.2	90.9
Gas	1	(5)	5	(9)	1.0	Referent		
Missing*	-	-	1	(2)	-	-	-	-
Kitchen location								
Outside	1	(5)	4	(7)	0.7	0.40	0.04	7.7
Inside	19	(95)	53	(91)	1.0	Referent		
Missing*	-	-	1	(2)	-	-	-	-
Windows								
Present	8	(40)	27	(47)	0.8	0.61	0.3	2.2
Absent	12	(60)	31	(53)	1.0	Referent		
Missing*	-	-	-	-	-	-	-	-
Doors								
2+	18	(90)	45	(78)	2.6	0.33	0.5	12.7
<2	2	(10)	13	(22)	1.0	Referent		
Missing*	-	-	-	-	-	-	-	-
Type of outer walls								
Sticks, sticks and planks	9	(45)	11	(19)	3.9	0.02	1.3	11.7
Concrete, planks only	10	(50)	47	(81)	1.0	Referent		
Missing*	1	(5)	-	-	-	-	-	-
Type of outer roof								
Thatch	15	(75)	43	(74)	1.1	0.94	0.3	3.4
Metal/rubber rye	5	(25)	15	(26)	1.0	Referent		
Type of floor								
Dirt	13	(65)	37	(64)	1.1	0.92	0.4	3.1
Concrete/wood	7	(35)	21	(36)	1.0	Referent		
Missing*	-	-	-	-	-	-	-	-
Other animals								
Present	14	(70)	47	(81)	0.6	0.31	0.2	1.7
Absent	6	(30)	11	(19)	1.0	Referent		
Missing*	-	-	-	-	-	-	-	-

*No observations were recorded in the census ‘-‘ represents no value.

Table 8

Frequency distributions, unadjusted odds ratios and 95% confidence limits (95% CL) for risk factors examined in relation with malaria case and non-malaria control houses, Red Bank, 1998

Variable	Cases n=20 #	(%)	Controls n=58 #	(%)	Odds Ratio	P-value	95% CL	
Horse(s)								
Present	3	(85)	11	(19)	0.7	0.25	0.04	7.7
Absent	17	(15)	47	(81)	1.0	Referent		
Missing*	-	-	-	-	-	-	-	-
Cat(s)								
Present	6	(30)	7	(12)	3.1	0.07	0.9	10.8
Absent	14	(70)	51	(88)	1.0	Referent		
Missing*	-	-	-	-	-	-	-	-
Dog(s)								
Present	10	(50)	20	(35)	1.9	0.22	0.7	5.3
Absent	10	(50)	38	(66)	1.0	Referent		
Missing*	-	-	-	-	-	-	-	-
Chicken								
Absent	7	(35)	12	(21)	2.1	0.20	0.7	6.3
Present	13	(65)	46	(79)	1.0	Referent		
Missing*	-	-	-	-	-	-	-	-
Grass around house (%)								
>50	7	(35)	22	(38)	1.3	0.80	0.3	5.9
16 – 50	10	(50)	23	(40)	1.7	0.50	0.4	7.5
≤15	3	(15)	12	(21)	1.0	Referent		
Missing*	-	-	1	(2)	-	-	-	-
Shrubs around house (%)								
>15	13	(65)	32	(55)	0.70	0.50	0.2	2.0
≤15	7	(35)	25	(3)	1.0	Referent		
Missing*	-	-	1	(2)	-	-	-	-
Trees around house (%)								
>15	13	(65)	39	(67)	0.90	0.80	0.3	2.5
≤15	7	(35)	18	(31)	1.0	Referent		
Missing*	-	-	1	2	-	-	-	-
Water around house (%)								
Present	5	25	9	16	1.8	0.40	0.5	6.1
Absent	15	75	48	83	1.0	Referent		
Missing*	-	-	1	2				
Bare ground around house (%)								
>10	8	40	31	53	0.60	0.30	0.2	1.6
≤10	12	60	26	45	1.0	Referent		
Missing*	-	-	1	2				

* No observations were recorded in the village census. '-' represents no value.

Table 9
Final stepwise logistic regression model indicating variables predicting malaria status in houses in San Martin

Variable	β	Standard error	OR	P-value	95% Confidence Interval	
Distance to stream (meters)	0.01	0.003	1.01	0.02	1.001	1.01
Number of years a case in past 4 years	0.70	0.30	2.0	0.02	1.1	3.6
Male occupants	0.35	0.20	1.4	0.04	1.0	2.0
Intercept	-5.82	1.20				

Table 10
Final stepwise logistic regression model indicating variables predicting malaria status in houses in Red Bank

Variable	β	Standard error	Odds Ratio	P-value	95% Confidence Interval	
Type of outer walls						
Sticks, sticks/planks	2.01	0.74	7.5	0.01	1.7	32.1
Concrete/planks	-	-	1.0	-	-	-
Missing*						
History of being a case in past 4 years	0.84	0.32	2.3	0.01	1.3	4.4
Female occupants per house						
5+	1.35	0.72	3.9	0.06	0.9	15.8
3 – 4	0.09	0.93	1.1	0.90	0.2	6.8
0 – 2	-	-	1.0		Referent	
Intercept	-3.79	0.90				

*No observations were recorded in the village census ‘-‘ represents no value

Table 11

Numbers and proportions of households that had 50% or more of malaria cases in San Martin and in Red Bank

	Households* with ≥50% malaria cases	Malaria cases	
	n (%)	n (%)	Total**
San Martin			
1993	13 (6)	51 (51)	101
1994	17 (8)	46 (52)	89
1995	16 (8)	46 (50)	92
1996	9 (4)	22 (50)	44
1997	8 (4)	16 (50)	31
1998	7 (3)	8 (53)	15
Red Bank			
1993	9 (9)	50 (51)	98
1994	5 (5)	19 (56)	34
1995	7 (7)	36 (54)	67
1996	12 (12)	59 (50)	117
1997	7 (7)	6 (53)	38
1998	5 (5)	11 (54)	11

* The Vector Control Program (VCP) surveyed 204 households in San Martin and 98 households in Red Bank.

**Total malaria cases that were linked to households surveyed by the VCP in the two villages.

Table 12
Numbers of three primary vectors collected in indoor-outdoor human landing collections in San Martin and Red Bank during August 21, 1996 through November 14, 1997*

	Indoors	Outdoors	Total
San Martin			
Anopheles albimanus	5	30	35
Anopheles darlingi	-	1	1
Anopheles vestitipennis	-	1	1
Red Bank			
Anopheles albimanus	2	6	8
Anopheles darlingi	16	37	53
Anopheles vestitipennis	-	-	-

*Eleven two-hour evening human landing collections were conducted in San Martin and ten two-hour evening human landing collections were made in Red Bank.

Table 13
Mean human biting rate* (HBR), by season**, for three potential vectors in San Martin, and in Red Bank

	HBR_{DRY}	HBR_{WET}
San Martin		
Anopheles albimanus	0.20	2.10
Anopheles darlingi	-	0.10
Anopheles vestitipennis	0.10	-
Red Bank		
Anopheles albimanus	0.11	0.50
Anopheles darlingi	1.30	2.50
Anopheles vestitipennis	-	-

*Human biting rate = No. of *Anopheles*/No. of collectors per site/two-hour landing collection
**Wet season: July through December; dry season: January through June

Table 14
Malaria incidence rate (per 1000 population), by age groups in male and female house occupants, in San Martin and Red Bank

	Females				Males			
Age group (years)	*P. falciparum* cases	Malaria cases	Population	Incidence	*P. falciparum* cases	Malaria cases	Population	Incidence
San Martin								
0-4	-	-	95	0	-	3	78	39
5-9*	-	3	95	32	-	5	106	47
10-14	-	1	93	11	-	8	84	95
15-19	-	1	63	16	-	2	70	29
20-24	1	2	43	47	-	-	45	0
25-29	-	-	40	0	-	1	43	23
30-34	-	-	36	0	-	1	32	31
35-39	-	1	36	28	-	2	29	69
40-44	-	1	21	48	-	-	30	0
45+	-	-	37	0	-	-	58	0
All ages	1	9	559	16	-	22	575	38
Malaria incidence	for males and females			27.3				
Red Bank								
0-4	-	8	58	138	1	8	57	140
5-9	-	2	54	37	1	3	64	31
10-14	-	3	42	71	-	2	40	75
15-19	-	1	32	31	-	1	27	37
20-24	-	3	27	0	-	-	25	0
25-29	-	-	23	44	-	1	17	9
30-34	-	-	12	0	-	1	27	0
35-39	-	1	8	250	-	1	8	250
40-44	-	1	9	0	-	-	7	0
45+	-	-	22	91	-	2	31	65
All ages	-	19	287	66	2	19	303	63
Malaria incidence	for males and females			63.7				

*Two villagers were missing gender information.

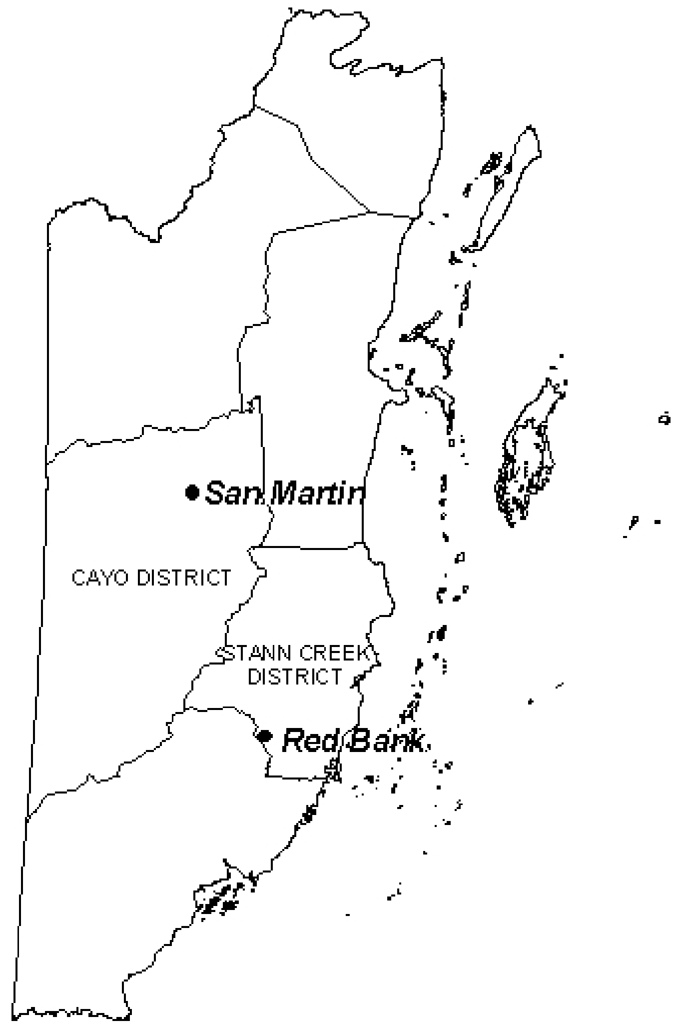

Figure 1: Location of San Martin, Cayo District and Red Bank, Stann Creek District in Belize, Central America

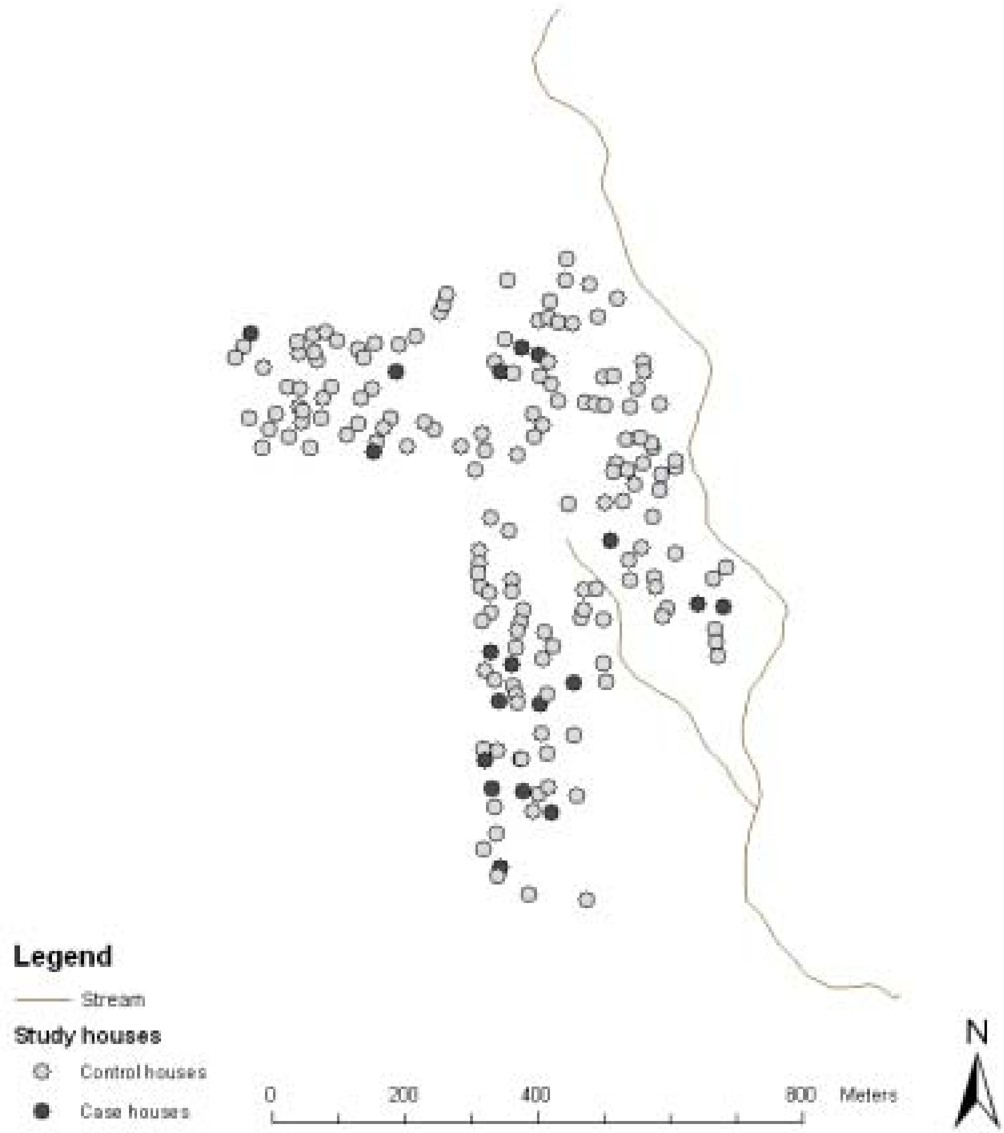

Figure 2: Study houses, by malaria case and control status in 1997, in San Martin

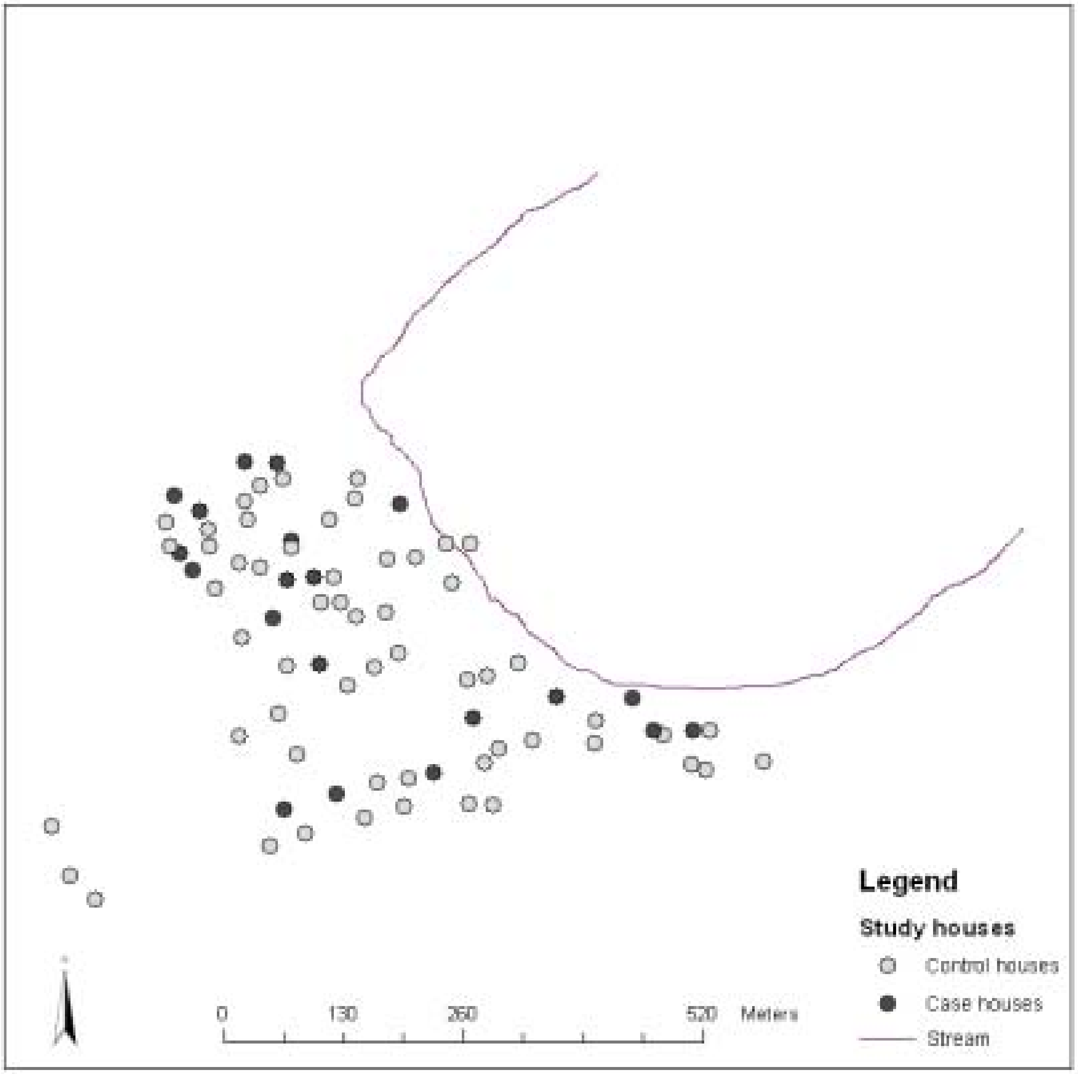

Figure 3: Study houses, by malaria case and control status in 1997, in Red Bank

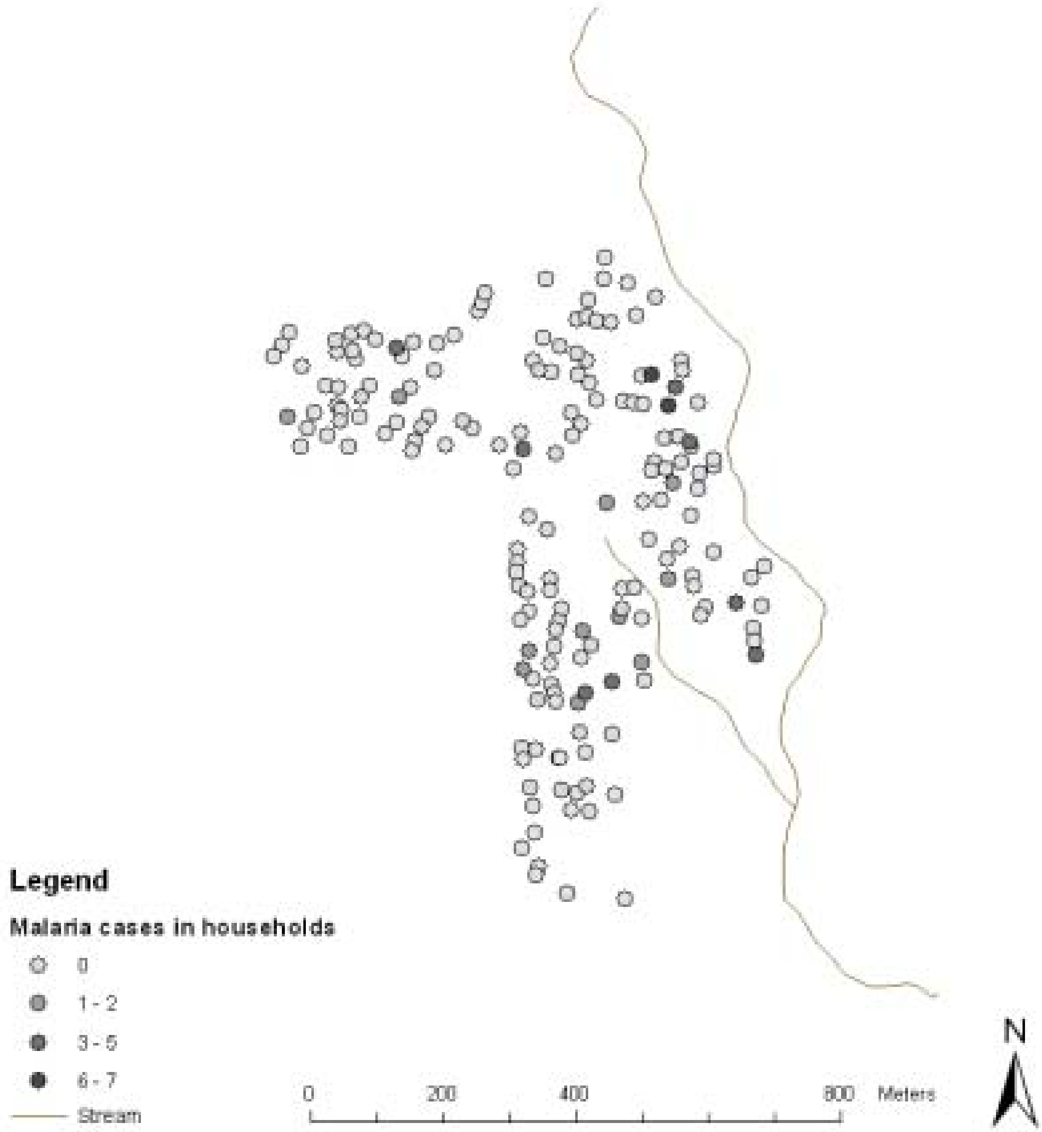

Figure 4: Four to 8% of households in San Martin had 50% or more of malaria cases during 1993 through 1998

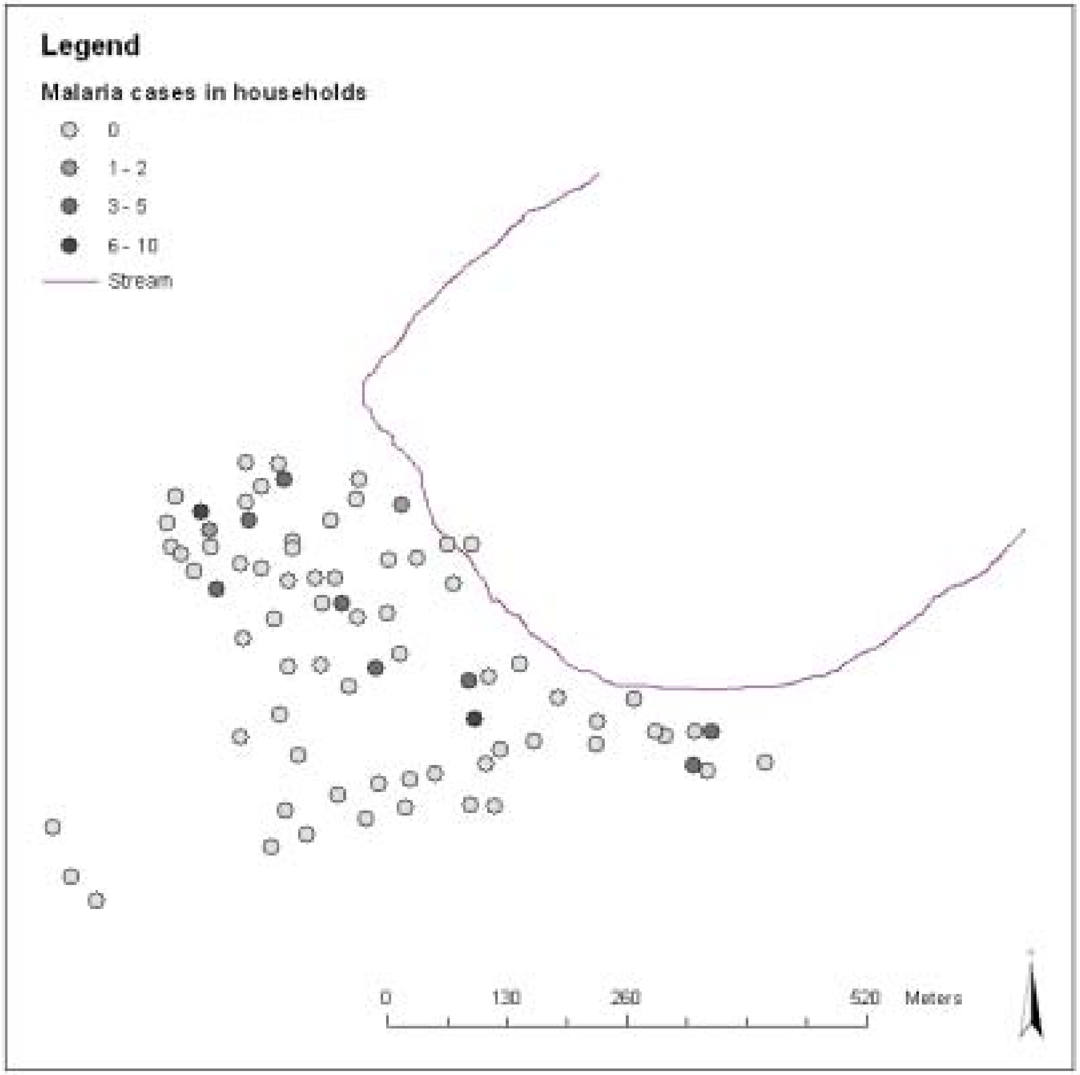

Figure 5: Five to 15% of Red Bank households had 55 to 63% of malaria cases during 1993 through 1997

Chapter 6

CONCLUSION

The objective of this dissertation was to describe the epidemiology of malaria in Belize over a 10-year period and determine if environmental factors influenced the incidence of malaria at macro- and micro-scales. The analyses were retrospective in nature and involved multiple factors. Data were obtained from several sources, which mostly were collected for purposes other than the specific aims of the studies in this dissertation. As a result, issues such as missing data, data gaps, and lack of information on important risk factors are inherent. This notwithstanding, the results do expand on previous studies of malaria transmission in Belize and offer added perspective to understanding the dynamics of malaria transmission in the country.

Examination of malaria incidence over a 10-year period indicated a spatial and temporal patterns existed in malaria incidence rates in the country. The southern and central areas of Belize had consistently higher rates of malaria than northern areas. Malaria incidence was highest during 1993 through 1996. A decrease and cessation in house spraying in the early 1990's allowed malaria rates to increase in the country by 40 percent in 1993 and rates increased again in 1994. Reduced residual house spraying was clearly one of the primary reasons for the increase in malaria rates where it ceased and for the variation in malaria burden in regions. A stratified spraying approach implemented in the latter part of 1996 decreased malaria rates by 41 percent in 1996. Areas of Belize in the northern districts bordering Mexico were sporadically sprayed during 1993 through 1995 (Bangs 1999).

Toledo District had the highest *Plasmodium vivax* incidence; whereas, Stann Creek District had the highest *P. falciparum* incidence. In Stann Creek District, the 0 to

4 and 35 to 44 age groups had the highest incidence of all age groups. *Plasmodium falciparum* incidence was highest in the transitional months preceding the wet season in Stann Creek.

Vector surveys conducted in villages in Cayo, Stann Creek, and Toledo Districts indicated that *Anopheles darlingi* was most common and abundant in Stann Creek District; whereas, *An. albimanus* was most common and abundant in the other two districts. The high incidence among young children in Stann Creek District indicates malaria transmission occurred in and around homes and therefore, within the villages. The vector surveys finding *An. darlingi* the most common vector in villages in Stann Creek District along with the epidemiology of *P. falciparum* incidence among very young children in Stann Creek District indicate that *An. darlingi* was the primary vector of *P. falciparum* in that district.

Preliminary results indicated malaria incidence differed geographically by season, type of vegetation, and proximity of villages to rivers or streams. Examination of data on weather and malaria incidence indicated that precipitation was associated with malaria transmission. Higher rainfall was associated with a higher malaria risk in villages. The relationships between rainfall and malaria incidence in microenvironments (represented by districts in this dissertation) indicated that the relationship seen for the country was especially significant in the districts of Cayo and Toledo where higher rainfall increased malaria risk in villages; whereas, the opposite was seen for Corozal and Orange Walk Districts. Examination of the relationship between vegetation and malaria incidence indicated that greater forest cover was associated with higher risk of malaria in villages. This relationship was strong in Belize, Cayo, and Stann Creek Districts.

The ecology of Cayo, Stann Creek, and Toledo Districts supports all three important vectors of malaria. In contrast, in Orange Walk and Corozal Districts, *An.albimanus* and *An. vestitipennis* are more common and *An. darlingi* populations are not (Bangs 1999, Rejmankova et al 1998, Rejmankova et al 1996). Northern Belize experiences significantly less rainfall, has more marshes and swamps and less forest cover, and fewer river systems than southern Belize. The variations in environmental factors determine the spatial distribution and abundance of the three vectors in Belize. The three vectors differ in vector compentency; *Anopheles darlingi* and *An. vestitipennis* are endophagic and anthropophilic and readily infected by *Plasmodium* (Grieco 2000, Roberts et al 2002). *Anopheles albimanus* is exophagic and zoophilic (Bangs 1999, Grieco 2000). Malaria risk coincides with the distribution of the three major vectors and the extent of human exposure to the three vectors. People in rural Toledo District live in substandard housing and have limited access to health care and education. Perhaps lifestyle and more open house construction result in these rural people having much greater exposure to vectors than other populations in the country. Additionally, lack of access to, and awareness of, primary health care possibly undermines the general health of this population. Aimpun (1999) conducted a prevalence study of intestinal parasites in a segment of the rural population of Toledo. He showed that the population had a significantly high prevalence of hookworm, *Strongyloides, Ascaris,* and other intestinal parasites. The prevalence was significantly higher than the rates reported for the country as a whole. Clearly, multiple factors such as vector ecology, and socio- economic, and behavioral factors of the rural population, contribute to the disparity in malaria burden in Toledo District as compared to other populations in Belize.

Environmental risk factors and malaria incidence were assessed in households in San Martin, Cayo District and Red Bank, Stann Creek District. In 1997, in San Martin, proximity of a household to a stream, number of male occupants in a household, and a history of malaria in a household were predictive of whether a household had malaria. In San Martin, malaria incidence was highest in males, particularly the 11 to 14 and 35 to 39 age groups. In Red Bank, in 1997, having a history of malaria in a household, construction of outer walls, and the number of females in a household were predictive of malaria in a household. The 0 to 4 year-old age group had the highest malaria incidence in Red Bank.

Vector surveys were conducted in 1997 and 1998 in both study villages. In San Martin, *An. albimanus, An. darlingi,* and *An. vestitipennis* were collected with *An. albimanus* being the most common vector present. In Red Bank, *An. darlingi* and *An. albimanus* were collected with *An. darlingi* being the most common vector. In 1997, Red Bank had an almost three-fold higher malaria rate than San Martin. Clearly, *An. darlingi* is the probable vector of malaria transmission in Red Bank and a more competent vector than *An. albimanus.*

Malaria cases clustered in both villages. In San Martin, during 1993 through 1998, three to eight percent of households produced 50 percent or more of malaria cases. Similarly, in Red Bank during 1993 through 1998, five to 12 percent of households produced 50 percent or more of malaria cases. Malaria preventive and control efforts targeting the traditionally high-risk households might significantly reduce the malaria burden in the community and translate into conserving human and financial resources of the malaria control program.

Malaria transmission is not randomly distributed. As demonstrated by previous studies in Belize and in the results of this work, it is influenced by vector ecology, malaria control efforts, environmental factors, human lifestyles, and standards of living. An understanding of the dynamics of malaria transmission in a locale aids in developing effective, affordable, and sustainable control measures. Further studies in vector competency and household risk factors are needed in Belize to expand our knowledge of malaria transmission in Belize. *Anopheles darlingi* is probably the most important vector of *P. falciparum* in Belize. Further studies are warranted to better understand the behavior and ecology of this vector in Belize. The role of migrant workers as gametocyte carriers needs further investigation, particularly in Stann Creek District. Risk factors varied in the two villages, which are situated in different geographic regions of Belize that were studied. Further studies are necessary to determine risk factors of malaria in other high incidence villages so as to aid with malaria control strategies at the household level. Additionally, a better understanding of the relationship between malaria risk and household factors in a village would be gained if residual spraying data for the household were available and included in the analysis.

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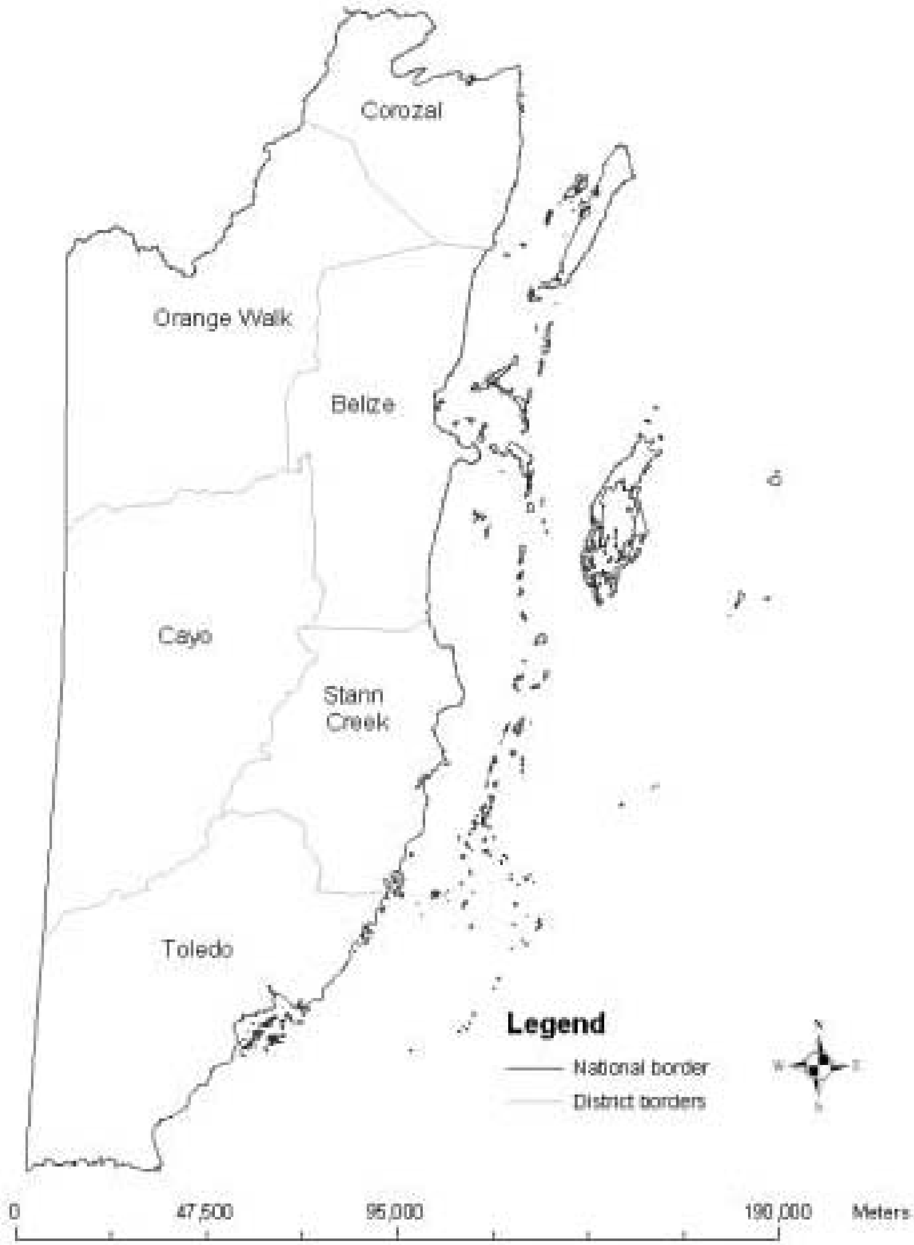
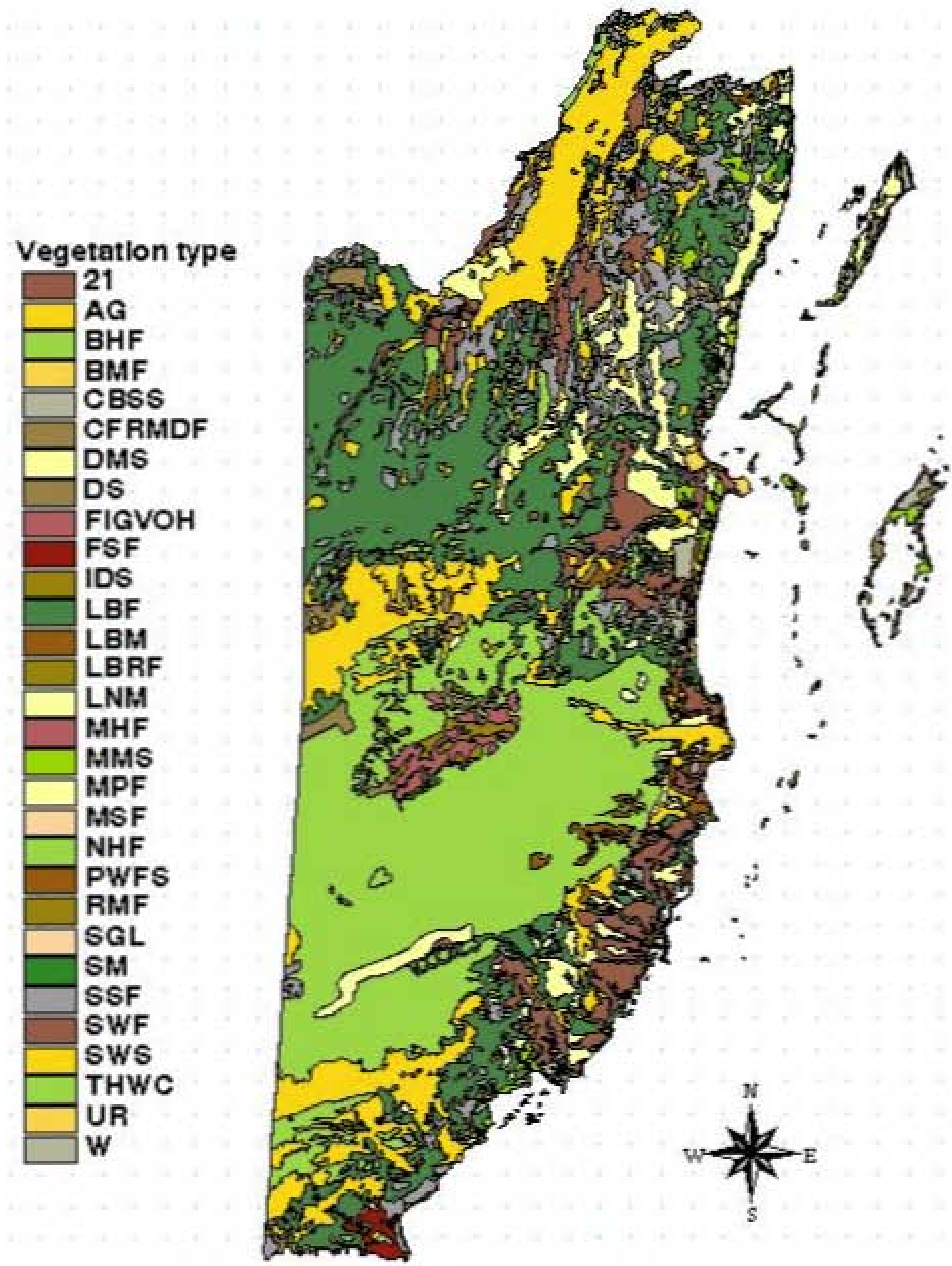
APPENDICES

Appendix 1
Map of Belize indicating the six administrative districts



Appendix 2
Vegetation map of Belize produced by Iremonger and Brokaw in 1994



Appendix 2 (cont.)

Key to the Iremonger, S., N.L.V. Brokaw vegetation map (published in 1995)

BELIZE VEGETATION TYPES

"Iremonger, S., N.L.V. Brokaw 1994 "

NON-WETLAND COMMUNITIES

AG	Agriculture
UR	Urban

FORESTS & SCRUBS

BHF	Broadleaf hill forests
DS	Disturbed scrub
FIGVOH	Fire-induced graminoid vegetation on hills
IDS	Inland well-drained shrubland
LBF	Lowland broadleaf moist evergreen seasonal forests
LBMSSF	Lowland broadleaf moist semi-evergreen scrub forest
LBRF	Lowland broadleaf rain forests
LNM	Lowland needle-leaf moist forest
MHF	Mixed hill forests
MPF	Montane palm forest
MSF	Montane scrub forest
NHF	Needle-leaf hill forests

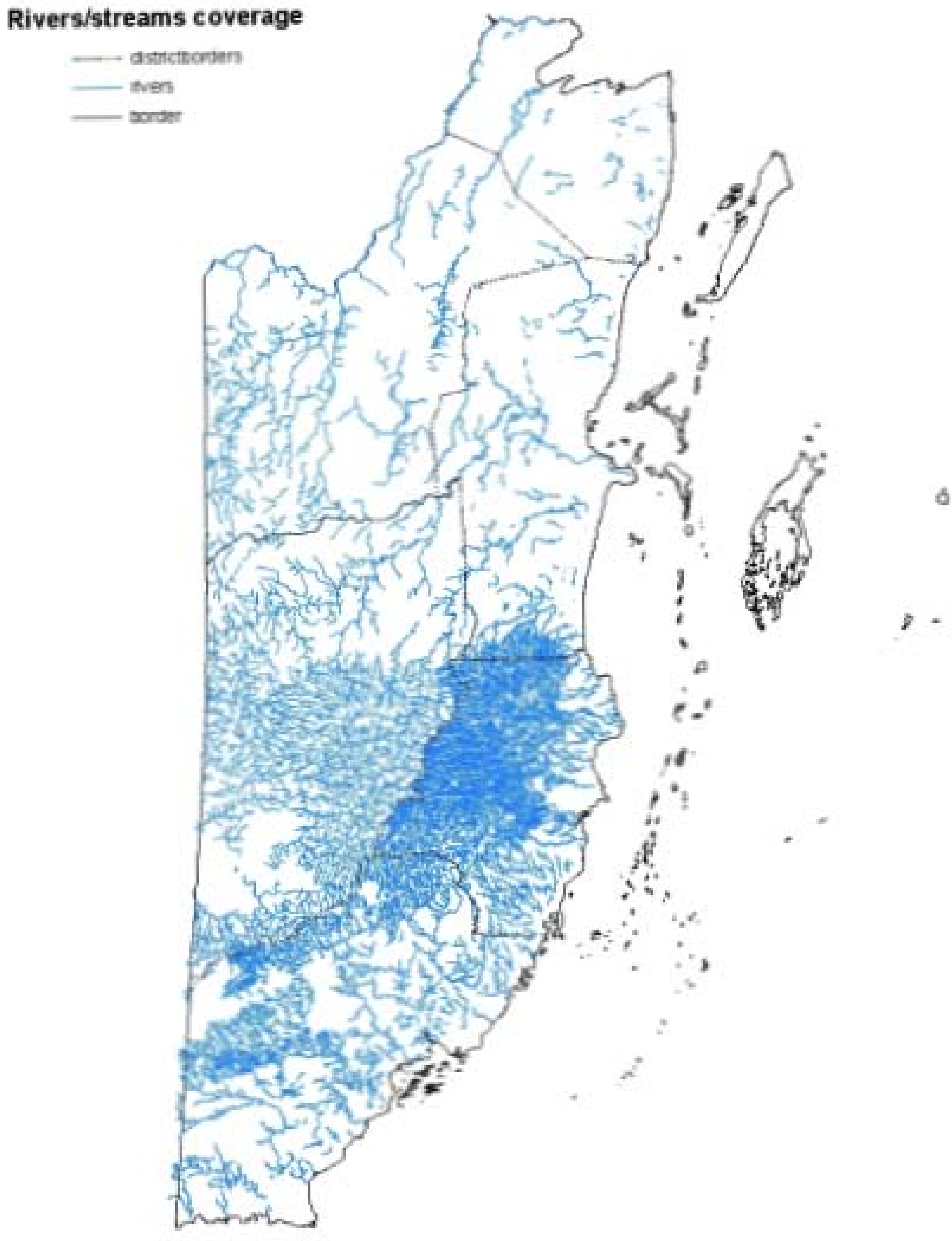
COASTAL COMMUNITIES

BMF	Basin mangrove forests
CBSS	Coastal beach sand scrubs
CFRMDF	Coastal fringe Rhizophora mangle-dominated forests
DMS	Dwarf mangrove scrub
MMS	Mixed mangrove scrub
SM	Salt marsh

WETLAND COMMUNITIES

FSF	Freshwater swamp forest
PWF	Permanently waterlogged freshwater scrubs
RMF	Riverine mangrove forests
RSSF	Riparian seasonal swamp forests
SGL	Swamp grassland
SSF	Seasonal swamp forests
SWF	Seasonally waterlogged fire-induced shrubland of the plains
SWS	Seasonally waterlogged scrub
THWC	Tall herb wetland communities
W	Water

Appendix 3
Rivers/streams coverage (Land Information Centre, Belize)



Appendix 4

Distribution of settlements positive (having 1 or more case of malaria) for malaria by district, by year, and by malaria species[1]

	Corozal	Orange Walk	Belize	Cayo	Stann Creek	Toledo
	n=47 (100%)	n=60 (100%)	n=53 (100%)	n=135 (100%)	n=46 (100%)	n=62 (100%)
P. vivax						
1989	31 (66)	21 (35)	12 (23)	32 (24)	16 (35)	31 (50)
1990	40 (85)	30 (50)	16 (30)	58 (43)	23 (50)	48 (77)
1992	36 (77)	37 (74)	20 (38)	74 (55)	27 (59)	44 (71)
1993	38 (81)	41 (82)	28 (53)	84 (62)	36 (78)	54 (87)
1994	42 (89)	40 (80)	29 (55)	82 (61)	34 (74)	55 (89)
1995	33 (70)	39 (78)	29 (55)	85 (63)	41 (89)	59 (95)
1996	31 (66)	26 (43)	24 (45)	72 (53)	38 (83)	54 (87)
1997	31 (66)	28 (47)	20 (38)	78 (58)	34 (74)	58 (94)
1998	26 (55)	17 (28)	12 (23)	59 (44)	30 (65)	51 (82)
1999	22 (47)	10 (17)	10 (19)	59 (44)	23 (50)	54 (87)
P. falciparum						
1989	0 -	3 (5)	3 (6)	11 (2)	0 -	3 (5)
1990	0 -	0 -	3 (6)	11 (2)	3 (7)	1 (2)
1992	5 (11)	3 (5)	1 (2)	26 (19)	9 (20)	7 (11)
1993	14 (30)	3 (5)	3 (6)	35 (26)	10 (22)	13 (21)
1994	15 (32)	4 (7)	5 (9)	37 (27)	14 (30)	13 (21)
1995	5 (11)	4 (7)	8 (15)	50 (37)	17 (37)	14 (23)
1996	6 (13)	6 (10)	12 (23)	27 (20)	21 (46)	19 (31)
1997	2 (4)	7 (2)	7 (13)	25 (19)	6 (13)	9 (15)
1998	0 -	4 (7)	3 (6)	27 (20)	2 (4)	4 (7)
1999	1 (2)	2 (3)	1 (2)	15 (11)	5 (11)	1 (2)

[1] The total number of settlements in the National Malaria Database is indicated for each district by 'n.' The numbers in parentheses indicate the proportion of positive (or %) of the total number of settlements in each district.

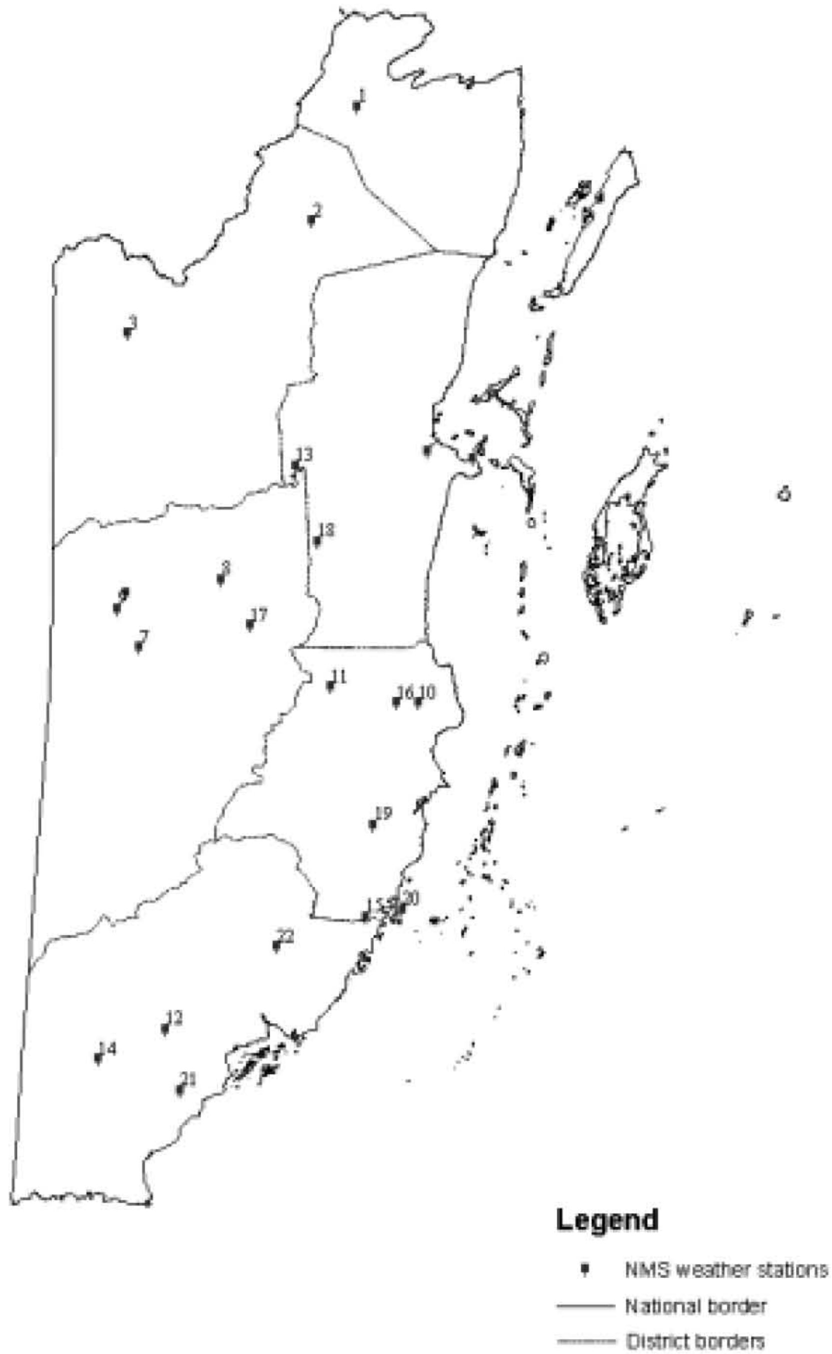
Appendix 5

Total *P. falciparum* and *P.vivax* cases, by age group, during 1989-1999 (except 1991)

Year	Infant	1 to 5	6 to 10	11 to 15	16 to 20	21 to 25	26 to 30	31 to 35	36 to 40	41 to 45	46 to 50	51 to 55	56 to 60	61 to 65	66 to 98	Total
P. falciparum																
1989	0	7	2	4	6	4	9	8	6	6	4	2	2	2	4	66
1990	0	8	3	2	4	6	6	5	4	2	0	0	0	0	4	44
1992	0	28	24	18	15	18	9	10	10	5	2	2	5	1	3	150
1993	2	50	50	26	27	31	16	8	13	8	8	4	4	2	5	254
1994	13	62	76	51	46	33	28	24	27	17	11	9	2	1	8	408
1995	4	82	72	47	63	52	41	21	36	16	14	9	6	4	5	472
1996	7	73	64	45	35	40	29	25	21	12	3	5	5	2	6	372
1997	1	9	9	14	15	13	9	12	8	11	2	4	6	2	6	121
1998	2	30	23	20	25	12	15	11	7	2	8	2	0	1	4	162
1999	1	7	5	9	4	8	3	4	4	0	0	1	0	0	0	46
Total	30	356	328	236	240	217	165	128	136	79	52	38	30	15	45	2095
P. vivax																
1989	5	479	455	423	422	328	270	228	175	112	75	38	44	20	45	3119
1990	6	459	474	415	390	338	255	181	128	75	60	43	30	17	44	2915
1992	6	747	731	704	705	530	442	297	239	157	98	75	43	50	65	4889
1993	124	1369	1281	1218	1041	843	652	491	382	263	180	122	97	68	98	8229
1994	129	1594	1709	1474	1271	934	789	632	475	336	211	90	100	62	86	9892
1995	150	1501	1417	1348	1108	853	673	528	411	269	222	106	74	78	90	8828
1996	99	899	870	746	674	504	381	271	224	167	99	69	57	32	60	5152
1997	100	769	579	527	478	359	298	220	166	111	88	49	46	33	53	3876
1998	44	347	245	204	216	178	147	92	94	60	37	28	22	17	22	1753
1999	46	379	291	200	184	133	148	112	80	66	44	29	20	16	42	1790
Total	709	8543	8052	7259	6489	5000	4055	3052	2374	1616	1114	649	533	393	605	50443

Appendix 6a

The locations of National Meteorological Service (NMS) weather stations in Belize



Appendix 6b

Data available, by date, for ground weather stations

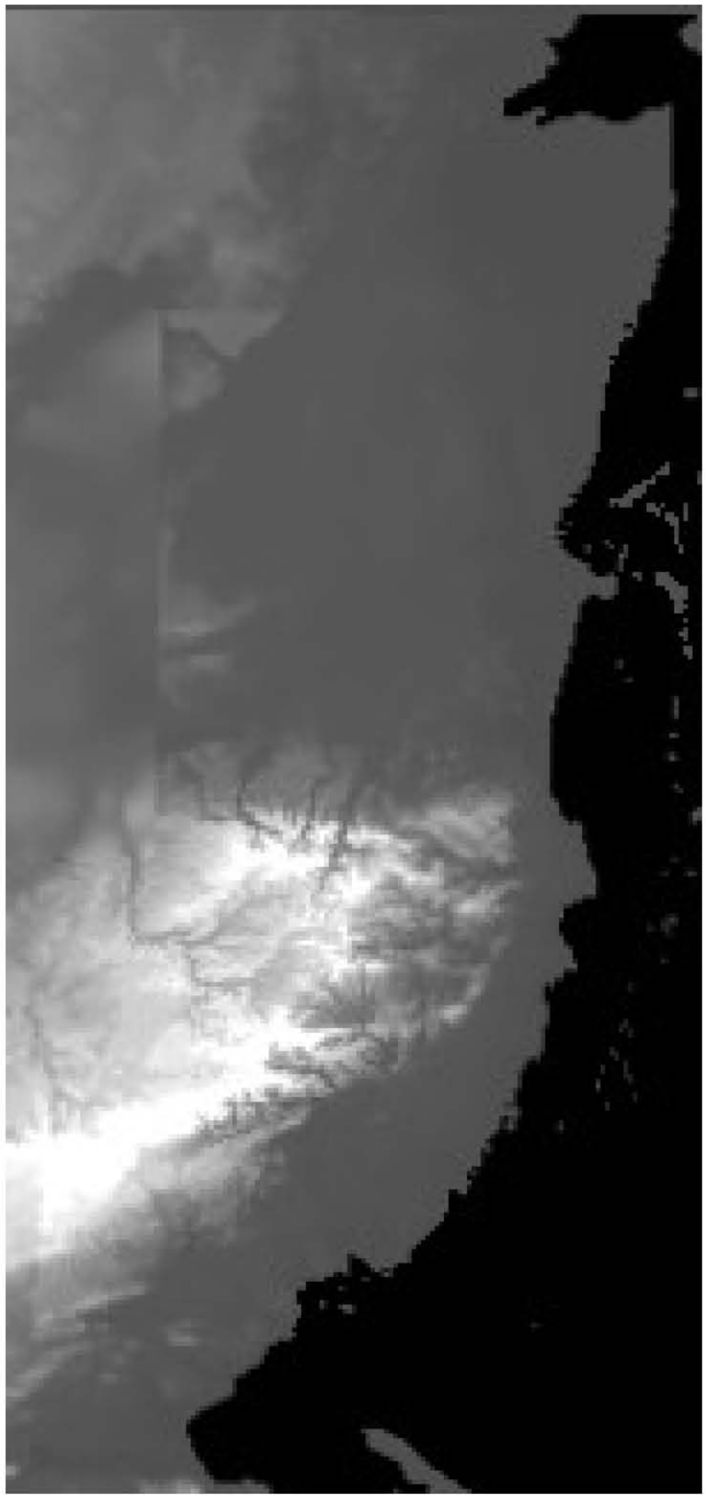
WEATHER STATION	Temperature (Highest of max, Lowest of min)	Rainfall (Daily 9:00 am)
1. Libertad	Jun.99-Jul.99 Oct.99-Dec.99 Feb.00-Nov.00	Sep.91-Jul.92 Sep.92-Mar.94 May 94-Oct.00
2. Tower Hill		Sep.91-Oct.00
3. Rio Bravo	Jan.92-Dec.95 Feb.96 Apr.96-Jun.96 Aug.96-Jan.97 Apr.97-Oct.97 Dec.97-Nov.00	Jan.95-Aug.95 Oct.95-Dec.95 Feb.96-Jun.96 Aug.96-Jan.97 Apr.97-Oct.97 Dec.97-Oct.00
4. St. John's College		Jan.92-Apr.92 Aug.92-Oct.96 Dec.96-Mar.99 May 99-Dec.00
5. Phillip Goldson Airport (PSWGIA)	Jan.89-Nov.00	Jan.89-Oct.00
6. Spanish Lookout		May 68-Jan.71 Mar.71-Dec.91 Feb.92-Apr.92 Jun.92-Feb.97 Apr.97-Dec.00
7. Barton Creek		Dec.92-Nov.97 Jan.98-Jan.00 Mar.00-Nov.00
8. Belmopan		Jan.74-Dec.74 Jan.76-Apr.86 Jun.86-Jul.87 Sep.87-Sep.89 Jun.90-Oct.94 Dec.94-Dec.00

WEATHER STATION	Temperature (Highest of max, Lowest of min)	Rainfall (Daily 9:00 am)
9. Central Farm	Jan.66-Dec.66 Apr.67-Dec.67 Feb.68, Apr.68 Jun.68-Mar.69 May 69-Aug.70 Nov.70-Feb.71 Apr.71-May 72 Jul.72-Dec.72 Apr.73-Sep.73 Mar.74-Aug.75 Jan.76-Sep.79 Feb.80-Oct.84 Jan.85-Dec.91 Mar.92-Dec.00	Jan.66-Sep.70 Dec.70-Jul.72 Oct.72-Dec.72 Apr.73-Jun.73 Jan.74-Oct.84 Jan.85-Dec.00
10. Melinda	Jan.73-Aug.79 Nov.79-Dec.79 Jan.81-Apr.82 May 85-Nov.87 Jan.88-Jul.89 Sep.89-Mar.91 May 91-Nov.92 Jan.93-Jun.95 Jun.97-Dec.00	Jan.73-Dec.79 Jan.81-Dec.82 Feb.84-Mar.85 May 85-Nov.87 Jan.88-Jul.89 Sep.89-Dec.00
11. Middlesex	Jan.90-Apr.91 Jun.91-Jul.91 May 92-Jun.92 Aug.92-Jan.93 Mar.93-Apr.93 Aug.93-Oct.93 Jan.94-May 94 Jul.94-Dec.94 Aug.99-Sep.99 Jan.00 May – Jun.00	Jan.66-Oct.90 Dec.90-Jul.91 Jun.92-Jan.96 Mar.96-Mar.99 May 99-Dec.00
12. Pomona	Oct.79-Oct.79 Nov.81-Nov.82 Apr.83-Jun.83 Oct.83-Jun.87 Sep.87-Oct.87 Jan.91-Mar.91 May 91-Aug.91 Nov.91 Jan.92-Jan.96 Jun.96-Mar.00 Jul.00 Nov.-Dec.00	Jan.66-Jun.72 Sep.72-Mar.77 Jun.77-Oct.87 Jan.88-Jul.91 Sep.91 Nov.91 Jan.92-Mar.00 Jul.00 Nov.-Dec.00

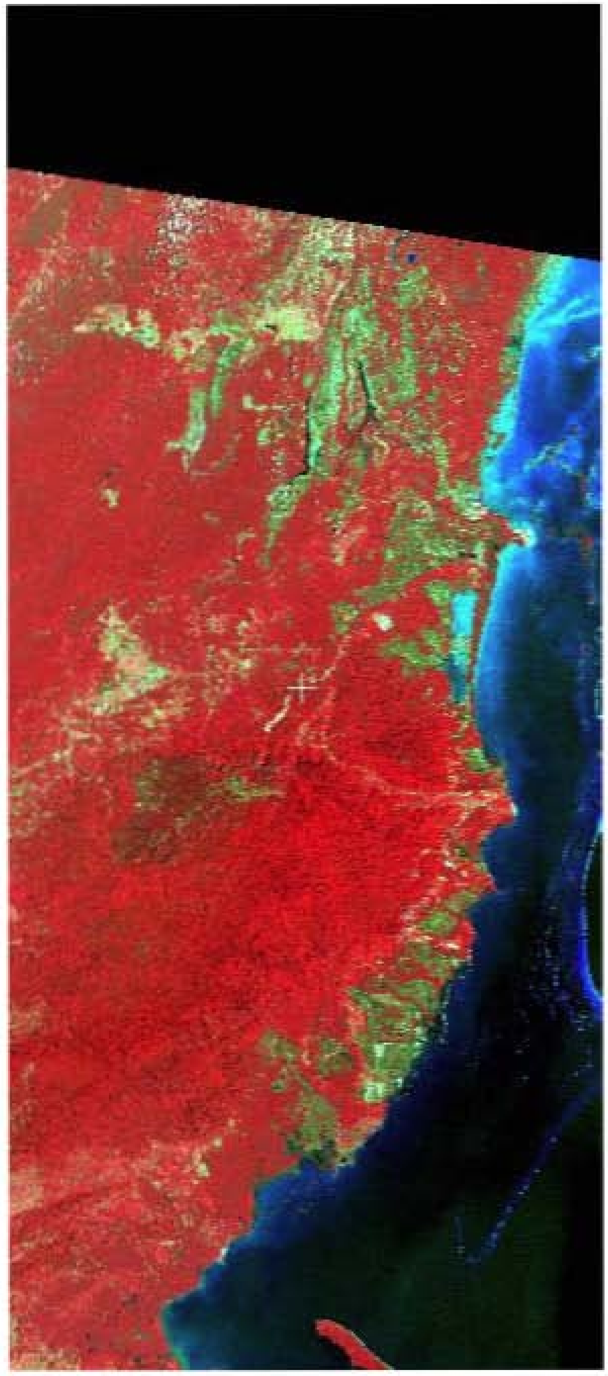
WEATHER STATION	Temperature (Highest of max, Lowest of min)	Rainfall (Daily 9:00 am)
13. Hershey1	Mar.95-Jul.96 Nov.98-Dec.99 Feb.00-Jul.00 Nov.00	Mar.95-Dec.00
14. Hershey2	May 93-Sep.93 Nov.93-Dec.00	Jan.01-Dec.00
15. Maya King	Dec.92 Feb.93-Oct.93 Jan.94-Jan.95 Oct.97-Feb.98 Jun.99-Jul.99 Oct.99-Dec.00	Dec.92-Apr.93 Jun.93-Mar.95 May 95-Jan.96 Mar.96-Jul.96 Oct.96-Jan.97 Mar.97-Jan.99 Mar.99-Jul.99 Oct.99-Dec.00
16. Rumpoint		Jan.93-Jul.93 Jan.94-Oct.96 Dec.96-Jan.97 Mar.97-Dec.00
17. Punta Gorda	Apr.76-Mar.80 May 80-Aug.92 May 93-Dec.93 Feb.94-Dec.94 Mar.95-Mar.96 May 96-Jan.97 Feb.98-Dec.00	Jan.66-Dec.00
18. Big Falls Plantation STATION 1 (near Punta Gorda)	May 95 Jul.95-Nov.95 Jan.96 Mar.96-Feb.97 Apr.97-Dec.00	Dec.84-Sep.90 Nov.90-Oct.92 Dec.92-Jul.93 Dec.93-Mar.97 May 97 Jul.97 Sep.97-Dec.97 Feb.98 Apr.98-Mar.99 May 99-Dec.00
19. Big Falls Plantation STATION 2 (in Belize District)		May 95 Jul.95-Feb.97 Apr.97-Jun.00 Aug.00-Dec.00
20. Blue Creek		Jan.85-Dec.86 Jun.87-Dec.00
21. Savannah	May 66-Jun.66 Nov.68-Nov.71 Jan.72-Mar.73 May 73-Jul.77	Feb.66-Jun.67 Nov.68-Nov.71 Jan.72-Mar.73 May 73-Jul.80

WEATHER STATION	Temperature (Highest of max, Lowest of min)	Rainfall (Daily 9:00 am)
22.Flores Farm(approx.)	Jan.78-Mar.80 Sep.80-Aug.81 Aug.82-Sep.82 Jan.92-Oct.92 Dec.92-Apr.93 Sep.93,Nov.93 Sep.94 Nov.94-Nov.95 Feb.98-Dec.00 Aug.98-Sep.98 Jan.99 Apr.00-Sep.00	Sep.80-Aug.81 Jan.82-Dec.00 Jan.98-Jan.99 Jun.99 Aug.99 Nov.99-Dec.99 Feb.00 Apr.00-May 00 Jul.00-Aug.00

Appendix 7
Global Land One-kilometer Base Elevation Data Set of Belize (National Geophysical Data Center/NGDC/NOAA)

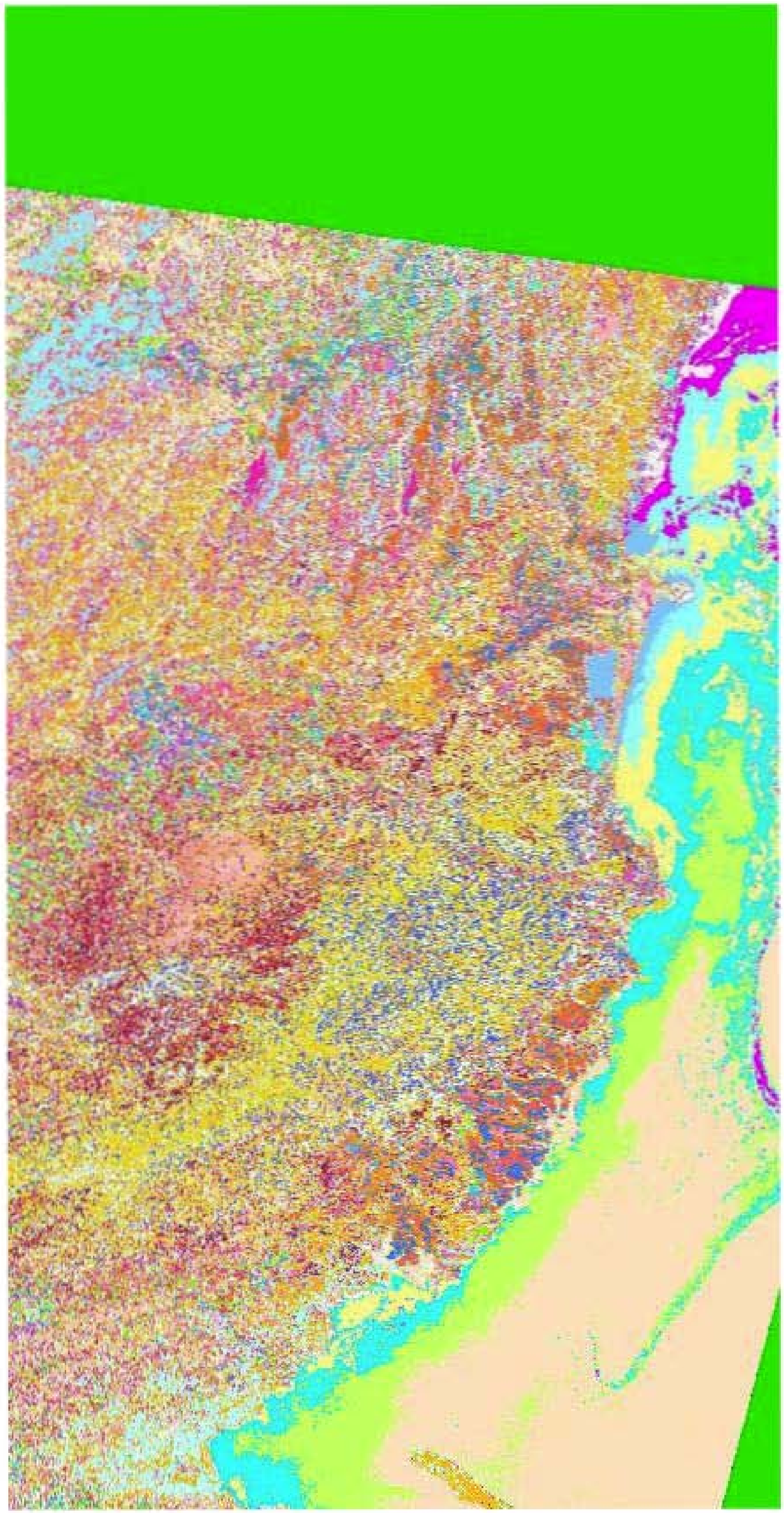


Appendix 8
Landsat image of Belize, March 1994



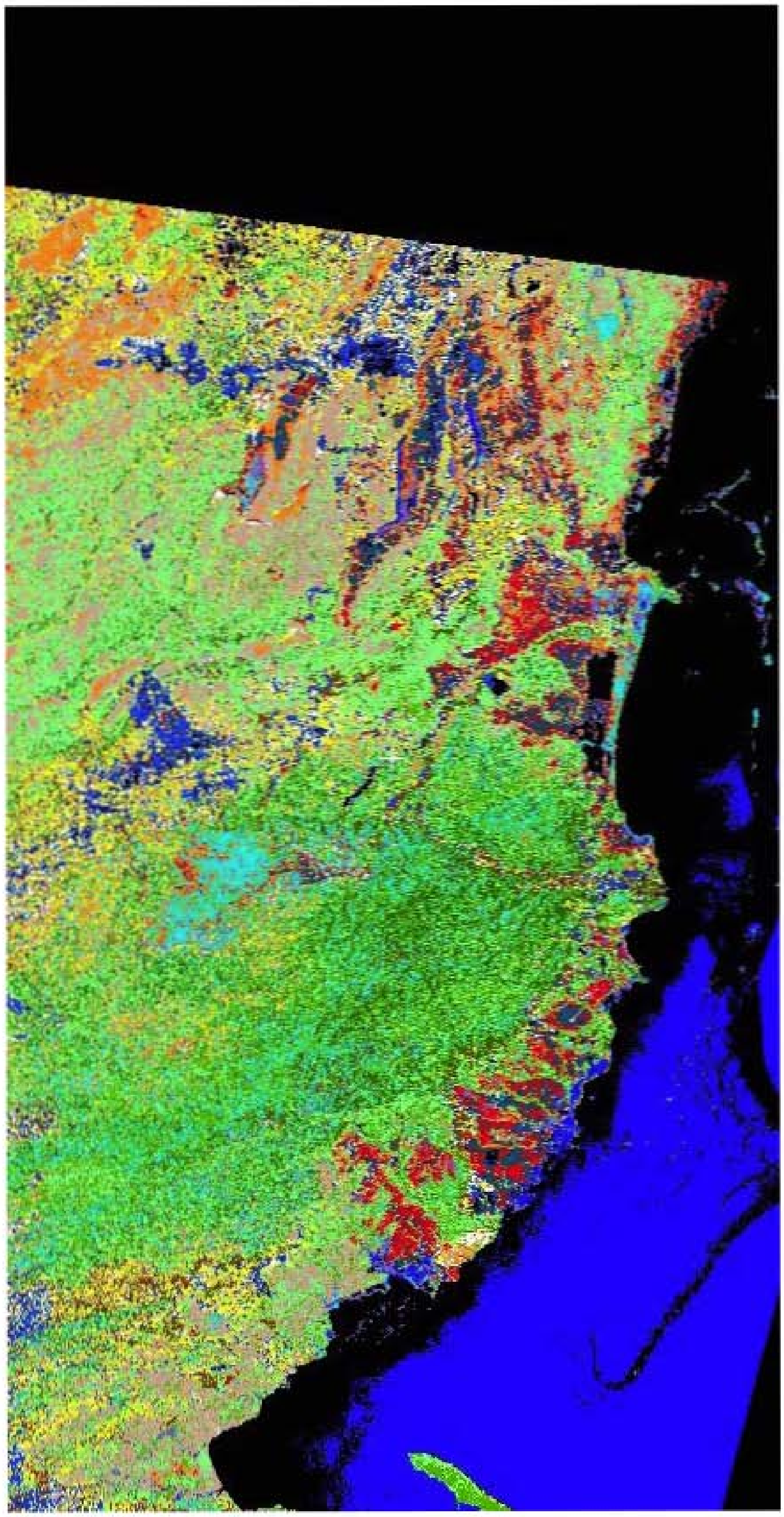
Appendix 9

Unsupervised classification (60-class, isodata) of 1994 Landsat image of Belize



Appendix 10

Unsupervised classification (30-class, isodata algorithm) of Landsat image



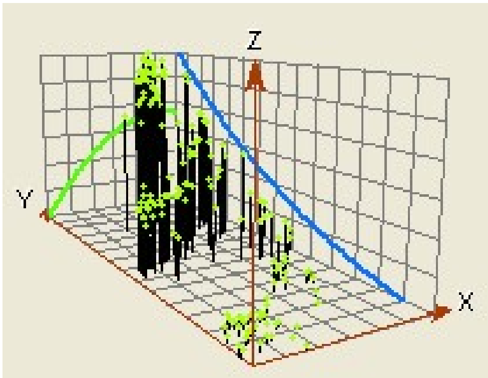
Appendix 11

Categorization in study of 17 classes field checked in 27 villages of Belize

Class	Field verification	Category used in analysis
2	Tall broadleaf forest	Forest
3	Water	Water
4	Dense broadleaf forest	Forest
5	Tall broadleaf forest	Forest
6	Agriculture	Agriculture
7	Tall broadleaf forest	Forest
8	Shrub forest	Forest
9	Red mangrove	Mangrove
10	Thick pine forest	Pine forest
11	Madeira palm	Savannah
12	Bare ground	Urban
13	Peri-domestic cultivation, rice fields	Domestic cultivation
14	Short grass savannah, *Eleocharis* marsh	Marsh
15	Farmland	Agriculture
16	Cleared land with grass	Agriculture
17	Sea	Sea
18	Grass/pasture/farmland/fallow field	Agriculture

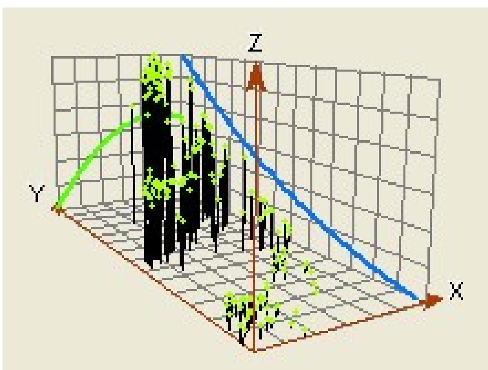
Appendix 12a

Trend analysis of weather variables (1993-1995), annual averaged daily maximum temperature



A1 : 1993

Annual averaged daily maximum temperatures during 1993-1995 increased in northerly (blue arrow) and easterly (green arrow) directions

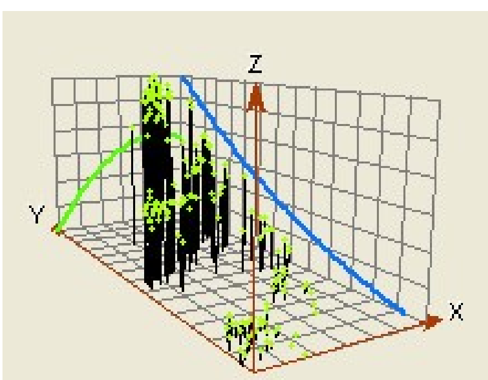


A2: 1994

LEGEND

Red arrows = x, y, z axes
X axis = eastern direction
Y axis = northern direction
Z axis = value of sample point

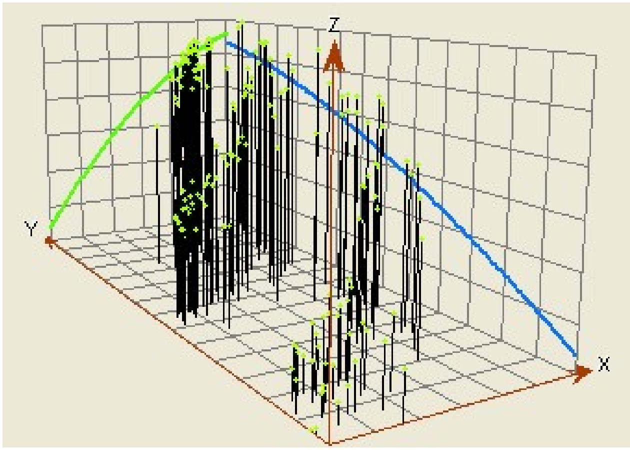
Black sticks = value of data point
Green curve = eastern trend of data
Blue curve = northern trend of data



A3: 1995

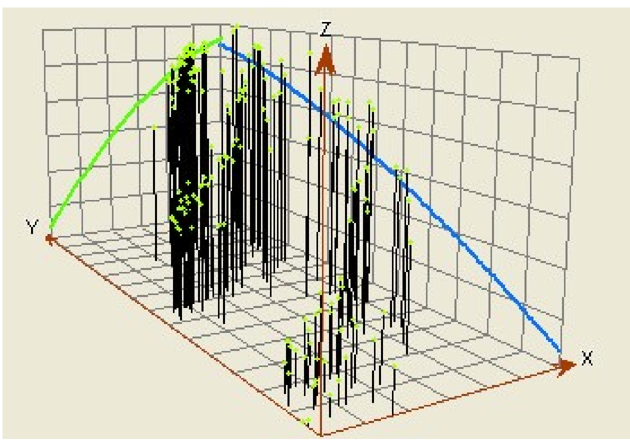
Appendix 12b

Trend analysis of weather variables (1993-1995), annual averaged daily minimum temperature

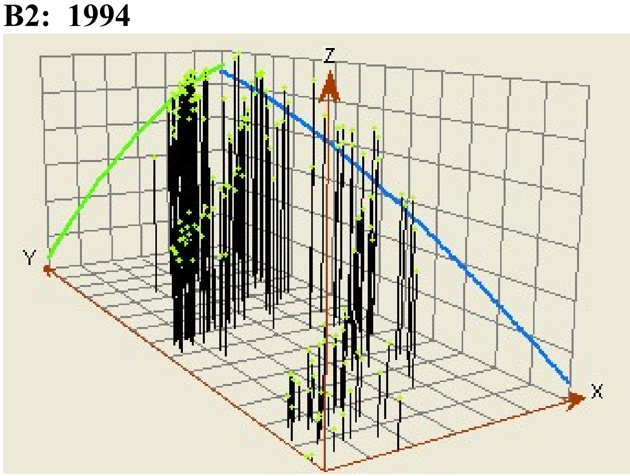


Annual averaged daily minimum temperatures during 1993-1995 decreased in southerly (blue arrow) and westerly (green arrow) directions

B1: 1993



B2: 1994



LEGEND

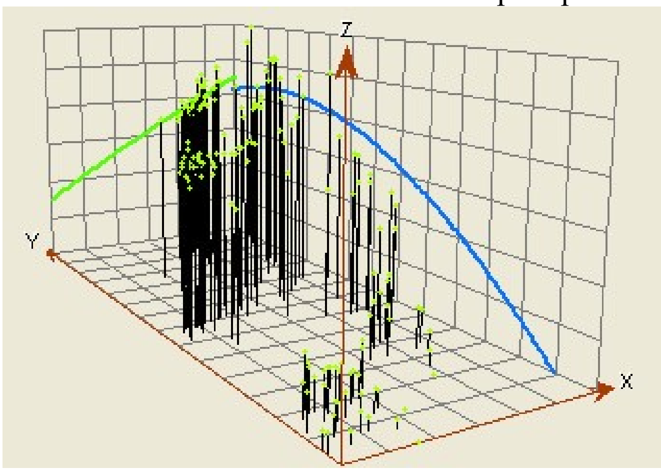
Red arrows = x, y, z axes
X axis = eastern direction
Y axis = northern direction
Z axis = value of sample point

Black sticks = value of data point
Green curve = eastern trend of data
Blue curve = northern trend of data
Green dots = location of data points

B3: 1995

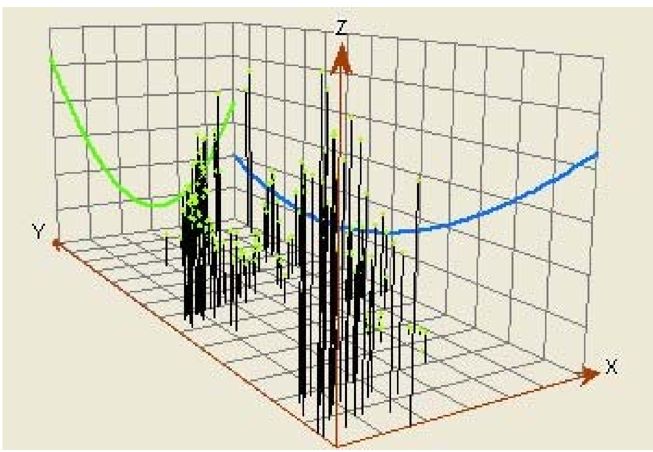
Appendix 12c

Trend analysis of weather variables (1993-1995), annual averaged daily total precipitation



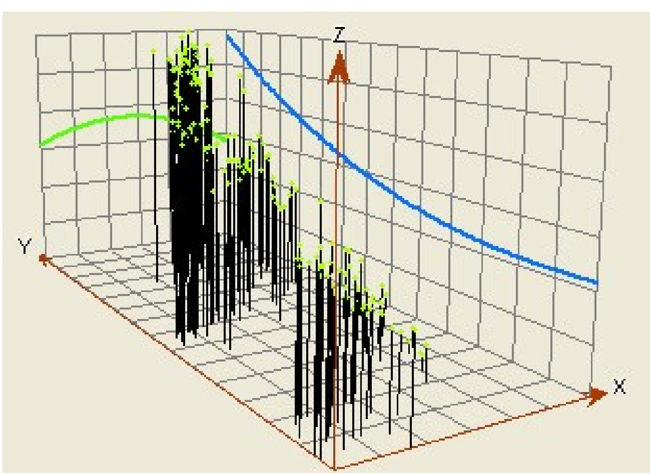
C1: 1993

Annual averaged daily total precipitation during 1993 decreased in southerly (blue arrow) and westerly (green arrow) directions



C2: 1994

Annual averaged daily total precipitation during 1994 increased in southerly (blue arrow) and westerly (green arrow) directions



C3: 1995

Annual averaged daily total precipitation during 1995 decreased in southerly (blue arrow) and easterly (green arrow) directions

LEGEND

Red arrows = x, y, z axes
X axis = eastern direction
Y axis = northern direction
Z axis = value of sample point

Black sticks = value of data point
Green curve = eastern trend of data
Blue curve = northern trend of data
Green dots = location of data point

Appendix 13a

SAS program written to perform univariate, bivariate, and multivariate analyses (country level)

```
dm 'log;clear' ;
dm 'out;clear';
options nocenter nodate;
proc import out=work.reg8wide
            datafile="C:\shilpa\study1\analyses\reg8lag.1mth.csv"
            DBMS=CSV REPLACE;
        GETNAMES=YES;
        RUN;
data reg8wide;
set reg8wide;

   offset=log(pop);
                slope0=(slope2=0);
                slope1=(slope2>0);
                if forestp=. then forestp=0;
                logurban=log(urbanp);

    array cases [3, 12] M93_1 - M93_12 M94_1 - M94_12 M95_1 - M95_12;
    array mintemp [3, 12] NT93_01 - NT93_12 NT94_01 - NT94_12 NT95_01 -
NT95_12;
    array maxtemp [3, 12] XT93_01 - XT93_12 XT94_01 - XT94_12 XT95_01 -
XT95_12;
    array rain [3, 12] R93_01 - R93_12 R94_01 - R94_12 R95_01 - R95_12;  /*all arrays
must be the same length here--if you need to save rwet92, we can figure out another
way*/


do j = 1 to 12;

                              month=j;
    case = (cases (1,j) + cases (2,j) + cases (3,j))/3;
    avnt = (mintemp (1,j) + mintemp (2,j) + mintemp (3,j))/3;
    avxt = (maxtemp (1,j) + maxtemp (2,j) + maxtemp (3,j))/3;
    ppt = (rain (1,j) + rain (2,j) + rain (3,j))/3;
                inci = (case / pop)*1000;
                lninci = log (inci);

    output;
end;

run;
```

UNIVARIATE ANALYSIS

```
proc means data=reg8wide;
var case pop avnt avxt ppt distance elev slope2 inldist domculp forestp agriculp
mangrovp marshp pineforp savannap urbanp waterp;
run;
```

BIVARIATE ANALYSES

```
proc genmod data=reg8wide;
class city_id;
model case = ppt  / dist=poisson link=log offset=offset lrci;
repeated subject=city_id / type=AR modelse;  /*ar(autoregressive):times that are closer
together have stronger correlations*/
run;

proc mixed data=reg8wide;
class city_id;
model ppt=avnt;
repeated / TYPE=AR (1) sub=city_id ;
run;
```

MULTIVARIATE ANALYSES

```
proc genmod data=reg7wide;
class city_id;
model case = ppt / dist=poisson link=log offset=offset lrci;
repeated subject=city_id / type=AR ;
run;
```

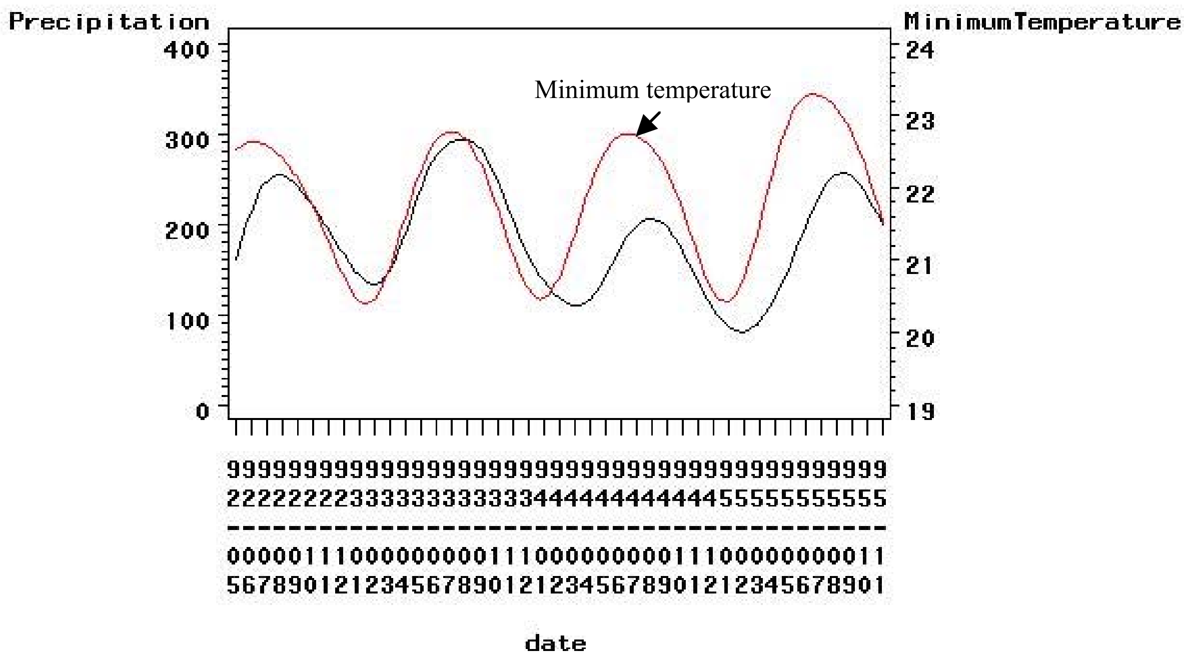
Appendix 13b

SAS program written to perform multivariate analyses (district level)

```
dm 'log;clear' ;
dm 'out;clear';
options nocenter nodate;
proc import out=work.reg8wide
            datafile="E:\study1\analyses\reg8lag.1mth.csv"
            DBMS=CSV REPLACE;
        GETNAMES=YES;
        RUN;
data reg8wide;
set reg8wide;

  offset=log(pop);
                slope0=(slope2=0);
                slope1=(slope2>0);
                if forestp=. then forestp=0;

    array cases [3, 12] M93_1 - M93_12 M94_1 - M94_12 M95_1 - M95_12;
    array mintemp [3, 12] NT93_01 - NT93_12 NT94_01 - NT94_12 NT95_01 - NT95_12;
    array maxtemp [3, 12] XT93_01 - XT93_12 XT94_01 - XT94_12 XT95_01 - XT95_12;
    array rain [3, 12] R93_01 - R93_12 R94_01 - R94_12 R95_01 - R95_12;  /*all arrays must be the same
length here--if you need to save rwet92, we can figure out another way*/

do i = 1 to 3;
do j = 1 to 12;
          yr=1992 + i;
                              month=j;
    case = cases (i,j);
    avnt = mintemp (i,j);
    avxt = maxtemp (i,j);
    ppt = rain (i,j);
                inci = (case / pop)*1000;
                lninci = log (inci);

    output;
end;
end;
run;
                              MULTIVARIATE ANALYSES
proc genmod data=reg8wide;
by distcode;
class city_id;
model case = ________ / dist=poisson link=log offset=offset lrci;
repeated subject=city_id / type=AR ;/* type=AR;  ar(autoregressive):times that are closer together have
stronger correlations*/
run;
```

Appendix 14

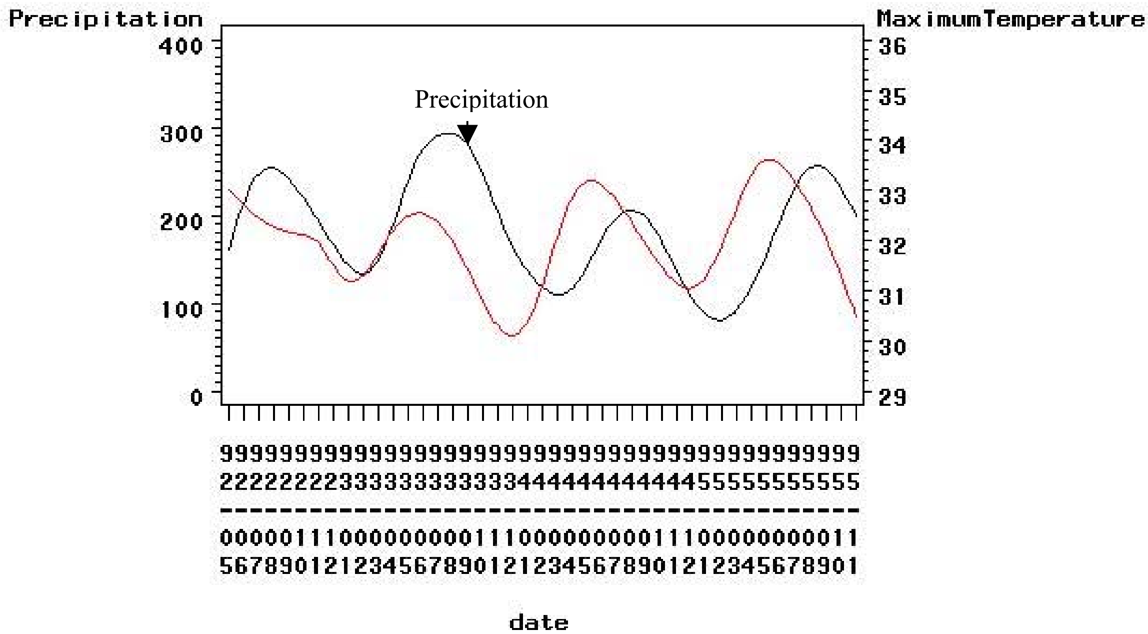
Smoothed plots of minimum and maximum temperatures (^0C) versus precipitation (mm) , by month of study period



A

Appendix 14

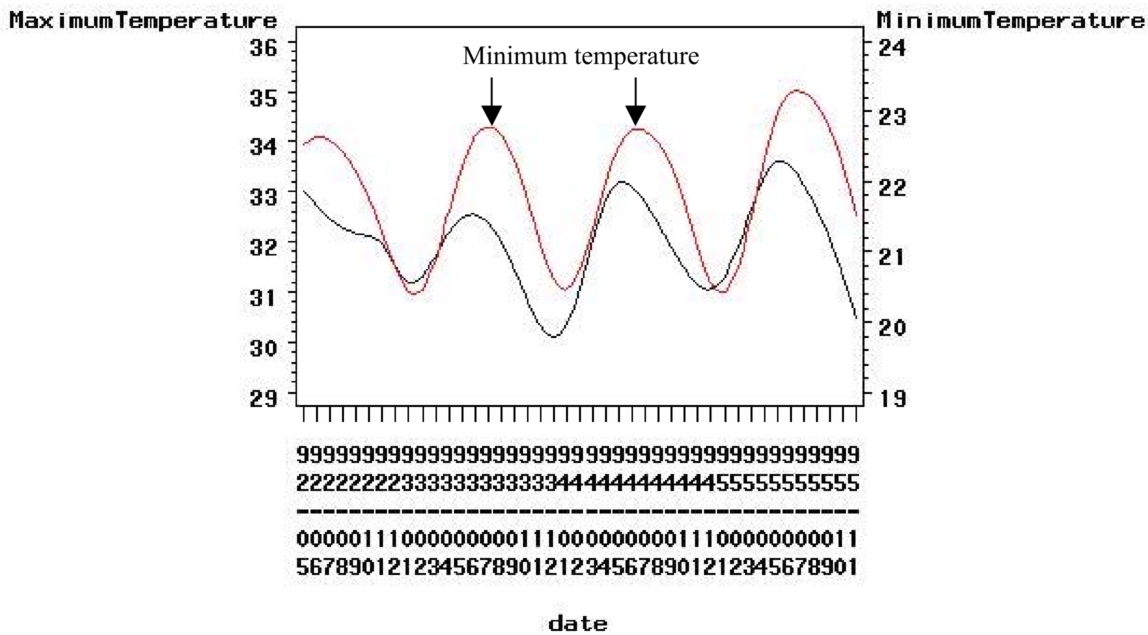
Smoothed plots of minimum and maximum temperatures (^{0}C) versus precipitation (mm) , by month of study period



B

Appendix 14

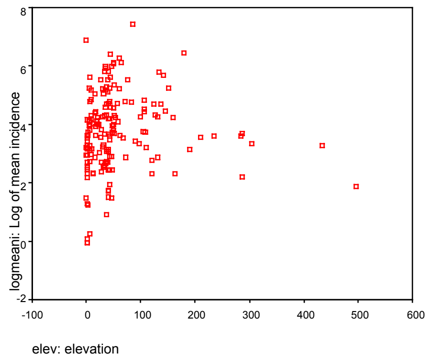
Smoothed plots of minimum and maximum temperatures (^{0}C) versus precipitation (mm) , by month of study period

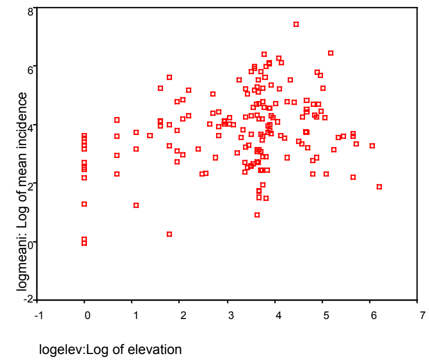


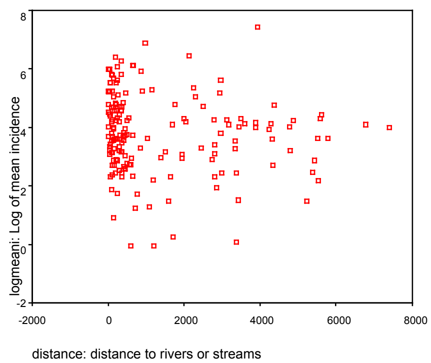
C

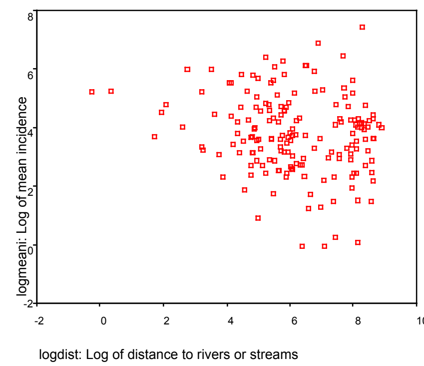
Appendix 15

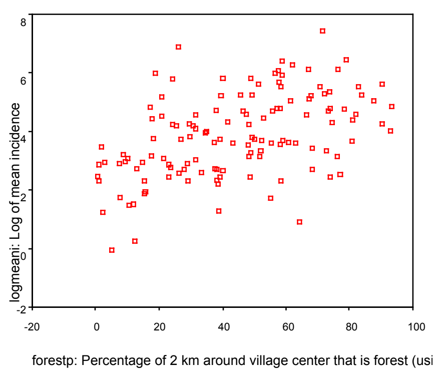
Plots of three environmental variables and their log transformation versus the log of malaria incidence





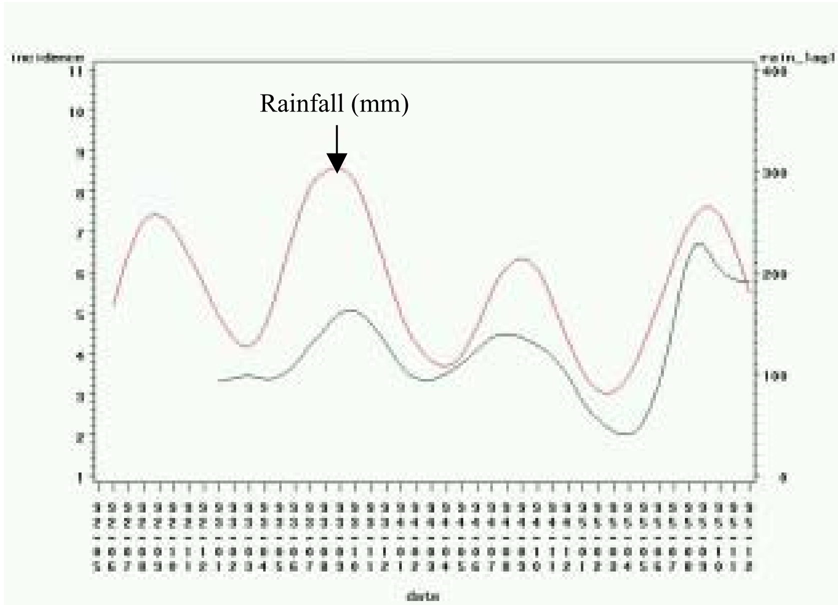




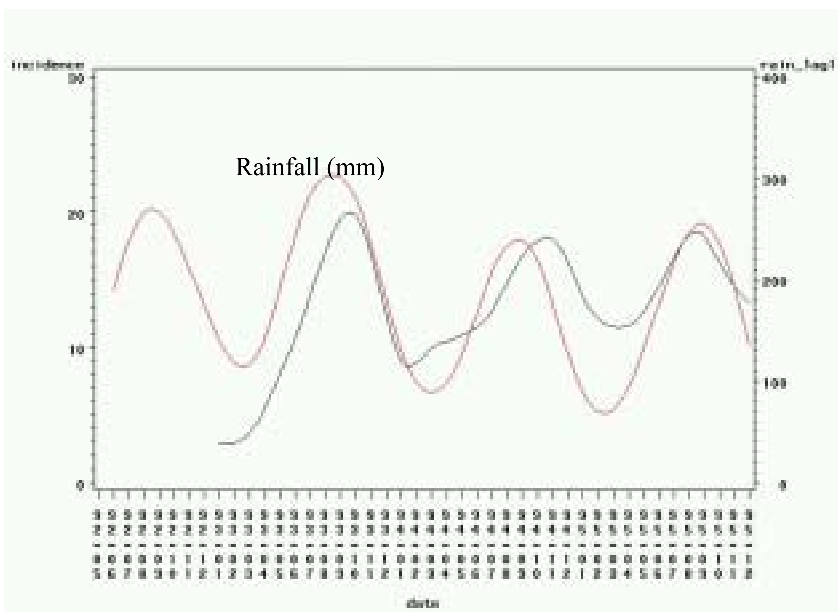


<u>Appendix 16</u>

Smoothed plots of precipitation lagged by one month versus malaria incidence for each month of the study period



Cayo District



Toledo District

Appendix 17

Spray schedule for the second cycle of 1997, Stann Creek District (Vector Control Program, Ministry of Health, Belize)

Schedule of House Spraying & Actual Performance by Locality

Year: 1997 **Cycle No. I**

Date: 3/3/1997 - 21/4/1997

Locality No.	Name	To Spray Houses	Population	Man Days Needed	Days Needed	Date of Spraying From	To	DDT (lbs) needed 75%	##	Dates Sprayed From	To	Day Used	Man Days Used	DDT (lbs) Used 75%	##	No. Sprayed House	Population	Total Existing House	Population	R	C	U	N	TOTAL	% coverage
626	Santa Rosa	43	168	4.7	0.7	3/3/1997	4/3/1997	39	3	3/3/1997	3/3/1997	3.8	0.6	36	0.44	35	171	41	184	0	0	0	13	13	90.4
627	San Roman	57	230	6.3	1	5/3/1997	5/3/1997	42	5	4/3/1997	4/3/1997	7.3	1.2	61	0.44	66	250	73	274	7	9	0	8	24	90.6
631	Georgetown	55	213	6.1	1	6/3/1997	7/3/1997	35	8	5/35/97	5/3/1997	8.6	1.4	20	42	78	319	86	325	5	1	0	0	6	95
630	Maya Mopan	61	290	6.7	1.1	10/3/1997	11/3/1997	56	5	6/3/1997	6/3/1997	6.6	1.1	58	5	60	292	63	308	0	12	0	4	16	88
639	Red Bank	110	464	12	2	12/3/1997	13/3/97	91	3	12/3/1997	12/3/1997	13.2	2.2	98	8	119	532	135	565	0	16	0	17	33	94
632	Cow Pen	499	1478	55	9.2	14/3/97	27/3/97	325	48	14/3/97	27/3/97	8	48.8	348	57	440	1701	467	1732	8	9	11	3	31	92
640	Trio Bladden H.	125	493	14	2.3	1/4/1997	3/4/1997	119	8	25/3/97	26/3/97	2	11.3	75	12	102	435	111	477	0	15		7	22	88
600	Community	63	253	7	1.1	7/4/1997	8/4/1997	47	26	7/4/1997	8/4/1997	0.7	4.7	82	30	55	256	64	297	26	0	0	15	41	84
603	Valley Rd. 2	145	665	16	2.6	9/4/1997	11/4/1997	91	72	7/4/1997	9/4/1997	3	17.4	33	66	132	707	157	867	36	19		28	83	97
605	Alta Vista	35	180	3.8	0.6	14/4/97	15/4/97	9	16	10/4/1997	10/4/1997	1	8.2	7	14	71	400	76	423	4	0	0	10	14	94
607	Pomona	175	585	19	3.2	16/4/97	21/4/97	54	88	16/4/97	18/4/97	3.4	20.6	94	11	186	882	198	935	32	2	0	19	53	100
647	San Pablo	11	65	1.2	0.2	12/3/1997	12/3/1997	11	0	13/3/97	13/3/97	0.2	1.2	11	0	11	65	11	65	0	0	0	0	0	100
602	Middlesex	52	268	5.7	0.9	9/4/1997	9/4/1997	12	26	9/4/1997	9/4/1997	1	5.7	12	26	52	268	52	268	0	0	0	0	0	100
644	Swasey	20	52	2.2	0.3	1/4/1997	4/4/1997	9	7	1/4/1997	1/4/1997	0.3	2.2	9	7	16	44	20	52	3	1	0	4	8	80

Appendix 18

Questionnaire used by the Vector Control Program team in the village surveys

Interviewer Initials:	Date(Day/Month/Year):	GPS Coordinates LAT: LONG:
House Number:	Village Code:	Village Name:

Occupant Names(Surname/First Name)	Age	Sex	Occupation	Location of job
1.__________ (Head of Household)	____	____	____	____
2.__________	____	____	____	____
3.__________	____	____	____	____
4.__________	____	____	____	____
5.__________	____	____	____	____
6.__________	____	____	____	____

How many years has present family inhabited house? __________	Where did occupants live before if new to this village? __________

Elevated House (circle correct answer) Y N	Kitchen (circle correct answer) Inside Outside Fire Gas	# of windows ______ Screened: Y N # Screened ______	# of doors______ Screened: Y N # Screened ______

Domestic Animals Horses Y N Cows Y N Pigs Y N Other ______	Walls In Planks Sticks Concrete Plaster Metal Other ______	Walls Out Planks Sticks Concrete Plaster Metal Other______	Roof In Metal Plaster Cardboard Wood Thatch Sticks Other ______	Roof Out Metal Plaster Cardboard Wood Thatch Sticks Other______

Surroundings within 20 meters around house Grass __________% Shrubs __________% Trees __________% Bare Ground __________% Water (River, pond, etc) __________%	# Walls ______ Were the inside walls sprayed with insecticide in 1997?	